The Evolution of Multiple Defences

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Lingzi Wang

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Abstract

This thesis aims to explore the evolution of multiple defences. Single defences have received considerable attention due to their specific effects in protecting organisms, but the research about multiple defences is relatively limited. Specifically, this thesis focuses on how the connection between defences is related to evolution (not the specific effect of protection in each defence). Chapters 2 and 3 focus on the connection between earlier and later defences using mathematical models. Chapters 4 and 5 focus on the connection between two synergistically acting defences, and their relationship with diversification rates using phylogenetics.

In Chapter 2, I explore the evolutionary reason for multiple defences and the trade-off between earlier and later defences. I find the conditions for multiple defences versus single defences and also found that, typically, the investment is more in earlier than later defences. In Chapter 3, I explore the defence phenotype variances in earlier and later defences in mutation-selection balance. I find that, typically, the earlier defence variance evolves to be less than the later defence variances, and I also find some factors that can influence the equilibrium variances. Both Chapter 2 and 3 show the relative importance of earlier defence to the later defences, due to their chances to use the defences. In Chapter 4, I study the coevolution between two synergistically acting defences, aposematism and group-living, and find that the root ancestor state is possibly group-living, which is slightly against intuition, as most previous research thinks otherwise. I also find the possible evolutionary dynamics of the four binary states from the ancestor till now and into the future, and the probability equilibrium values of the four states. In Chapter 5, I have extended the study of defences into a macroevolution point of view and study the association between defences and diversification rates. Here I have obtained further evidence to "escape and radiate"

hypothesis regarding the association between aposematism and faster diversification rates. I also find that group-living is positively associated with diversification rates, which is new in this area to my knowledge. The previous research about chemical defences in this topic is not consistent, which might be because chemical defences are usually deployed later, therefore, are less important for protection and can be more variable, as I have proposed in Chapter 2 and 3.

In all, the findings here imply that the connection between defences plays an important role in the evolution of multiple defences. This can help us to further understand the evolutionary reasons and patterns of multiple defences and their application in certain practical areas.

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This is the end of one journey, but it is also the start of the next. Will see.

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Chapter 1

Introduction

1.1 General background

Biological organisms need defences to protect themselves from different enemies in nature, such as predators, pathogens, and parasites. Many organisms are observed to have multiple defences (e.g. morphological, physical, chemical, behavioural, physiological defences) rather than only one defence. For example, some organisms have both constitutive (always present) and induced defences (produced in specific circumstances) [1]. Both constitutive and induced defences can take multiple forms as well. Examples of constitutive defences include cryptic appearance, thick epidermises, thorns, and toxins. Induced defences can be secreted sap (when some tissues are broken), closure of parts of the body (e.g. plant's leaf stoma) or chemicals.

Research on multiple defences is mainly focused on the following areas:

(1) The evolutionary reasons for multiple defences [2, 3], the trade-off between defences for multiple defences [2, 4, 5, 6].

(2) The different functions of multiple defences working against different enemies [7], or in different circumstances [5, 6, 8, 9, 10].

(3) The sequential deployment of multiple defences [2, 11].

(4) Synergistic effects between defences [3, 12, 13, 14].

(5) The applied use of multiple defences in non-biological areas [11].

In this introduction, I will first introduce (1)-(4) in order. I will also mention (1) in different deployed ways in multiple defences (2)(3)(4).

In the thesis, the second chapter will explore the evolutionary reasons for sequentially deployed defences and the trade-off between earlier and later defences(points 1 and 3 above). The third chapter will analyse the evolution of variances in earlier and later defences (points 1 and 3). The fourth chapter focuses on the synergistic effects and evolutionary mechanisms of two contrasting defences in the Macrolepidoptera Order (points 1 and 4). The fifth chapter will test the effect of defences on diversification rates of species (which is usually analysed in relation to one defence in the previous research [15, 16, 17, 18, 19, 20, 21], so I will analyse the question with two defences in order to offer more evidence to the question on this phenomenon).

1.2 The evolution of multiple defences

Nature usually selects the phenotypes that are most adaptive [22], and this principle applies to defence phenotypes as it does to other phenotypes. Through adaptive evolution, defences are assumed to evolve to be well "designed" and perform efficiently. For example, panther chameleons *Furcifer pardalis* can change their colour quickly and perfectly as their background changes [23]; some animals can even be transparent (e.g. glass squid – *Bathothauma lyromma* [24], glasswing butterfly – *Greta oto* [25]), and so appear invisible to certain predators.

Since multiple defences are so commonly observed in nature, an important question is why they are selected and preserved i.e. what are their benefits, compared to investing in a single "super-defence"? The general reasons for the evolution of multiple, rather than single, defences have been analysed in mathematical models [2, 3, 26, 27]. Two important aspects are usually included in the analyses in these models, namely fitness-related investment and damage costs.

(A) Fitness related investment: how effectively does the investment convert fitness resources into defences.

Investment in defences is regarded to have"allocation costs" that diminish the fitness resources, which could otherwise be applied to other fitness-enhancing activities [28]. The reason for investment in defences is that defences can protect organisms against enemy attack (predators, parasites, etc) [28]. The effectiveness of investment in defences is usually reflected in the probabilities that the invested defences can successfully hold to prevent enemies (instead of being breached by enemies) [2, 3, 26]. When investing in multiple defences, once one of the defences is breached, there is a chance that the other defences will hold up and the organism will be able to survive, compared to investing in only one defence. Therefore, the spread of investment in multiple, rather than single, defences might help to increase the chance of survival.

(B) Damage costs: the damage costs to victims during or after enemy attack.

The exposure or the failure of each defence to the enemies is considered to have a cost to the victims [28]. If there is only one defence, then the full damage costs will be to the victim (e.g. death) when this single defence is breached. However, when there exist several defences, the full damage cost can be spread into several parts, and there is a cost on each part when the corresponding defence is breached, so the cost could be less when only parts of the multiple defences are breached – the organism survives but some tissues are destroyed.

The existing models in the current literature usually combine the effects of both "fitness-related investment" and "damage costs", so as to analyse both the benefits and costs of defences. Different defence strategies can be compared using the models. For example, the models can be used to compare the strategies of multiple defences vs one single defence [2, 26, 27], or the trade-off between different defences [2, 3]. The use of"fitness-related investment" and "damage costs" in the analysis of the evolutionary reason for multiple defences will be mentioned in the later sections about the three ways in which multiple defences are deployed.

1.3 The ways in which multiple defences act

Multiple defences can be deployed in different ways, and three ways are often observed. First, multiple defences act individually in different circumstances (parallel-deployed). Second, multiple defences act individually in an order (sequentially-deployed). Third, multiple

defences act synergistically together (either parallel or sequentially). Here I will discuss three different ways that multiple defences are deployed. The evolutionary reason for multiple defences in each of these three deployed ways will also be shown using "fitness-related investment" and "damage costs".

1.3.1 Defences acting individually in different circumstances

Individually deployed defences

Multiple defences are usually used in different circumstances [29], for example, (a) towards different enemies [3, 30], (b) in different environments [8], or (c) in different stages in their lives.

(a) Prey can be targeted by more than one species of predator. At the same time, they might also suffer attacks from pathogens and parasites. Therefore, in the face of different enemies, multiple defences can be useful in protecting the victims from different alternative kinds of enemies.

(b) Some species use multiple defences in different environments (in different places they live, or seasons of the year, etc). For example, animals need to protect themselves in the environmental surroundings they inhabit, and their surroundings can be disparate in time or place, so they might need corresponding camouflage defences as the environment surrounding them changes. Countershading [10] is found in many animals (e.g. fish, birds, reptiles and mammals). Darker-coloured backs but paler-coloured underside protect them from observation by predators both under their body and above their body in the background of both the sky and the land respectively. Also, it can help to make them appear less solid and conspicuous, as normal (i.e. uniformly coloured) three-dimensional objects usually appear lighter on the top and darker on the bottom. The fur of the snowshoe hare (*Lepus americanus*) can adapt to turn white in winter and brown in summer, so as to hide against both the snowy background in winter and the soil brown background in summer [9]. *Rana pirica* tadpoles can develop predator-specific morphologies towards different types of predators and the survival rates are higher when the tadpoles' morphologies can adapt to specific types of predators [31].

(c) Some species use multiple defences in different stages of their lives, especially for those

metamorphosis species, or those species that change their living places in different life-times. For example, *Phigalia titea* caterpillars mimic wood sticks; adults are cryptic in the tree trunks or on the rocks and can fly away when discovered by predators [32]. Cicada nymphs(e.g. *Magicicada tredecassini*) dig holes to hide underground, and their adults live above grounds and have wings and camouflage colours as protection [33].

The evolution of individually deployed defences

Multiple defences evolve individually in their own respective circumstances and do not necessarily function in circumstances when other defences may be required. Organisms can, therefore, find themselves in danger when their defences are not specifically evolved to defend against a different type of enemy attack. Speed *et al.* [27] have used a model to explore the evolutionary reason for this type of multiple defences. The model, which includes "fitness-related investment", analyses the effect of coevolution between multiple toxicity defence traits in plants and multiple corresponding resistance traits in insects. It shows that plants are less likely to go extinct as the number of defence traits increases. Moreover, it shows that there is a trade-off among defences, which is also found empirically in direct defences (the release of hydrogen cyanide) and indirect defences (the emission of volatile organic compounds), and the amounts of investment are negatively related with each other [4].

1.3.2 Defences acting sequentially

Sequentially deployed defences

Sequential defences are also used independently, but they are carried out in order, one after another. The earliest defence in the sequence is first initiated when the victims are attacked by enemies, with the later defences inactive; however, when the earliest defences are breached by the enemies, the subsequent defences start to get work one by one to defend against further attacks. For example, constitutive defences usually function as the first line of defence, and induced defences usually function later as the second line of defence after

the constitutive defences have failed [1]. In the human immune system, physical defences (e.g. skin, hair) usually function first to prevent pathogens (e.g. bacteria and viruses) from entering the human body. Once these physical defences have been breached, the innate immune defences will respond immediately and generally against the invading pathogens [34]. If the innate immune defences cannot kill the pathogens, the subsequent adaptive immune defences will then be activated, so the specific memory cells (B cell and T cell) will respond and eliminate the specific kinds of pathogens [35, 36].

The evolution of sequentially deployed defences

One reason for the evolution of sequential defences is that attacks from predators can be sequential [37]. For example, predators can show a series of predation behaviours — detection, identification, approach, subjugation, consumption. Prey defences may have separate evolutionary trajectories towards the corresponding attacks at each phase [38]. Since the overall resources for the organisms' fitness and defences are limited, increasing investment in one defence may cause decreasing investment in the other defences (trade-off between defences) [22, 38]. For example, in the reed warbler *Acrocephalus scirpaceus*, the egg-rejection defence is observed to block the later defence of chick-rejection against the common cuckoo *Cuculus canorus*, but the same does not happen in the superb fairy-wren *Malurus cyaneus*, which chooses the chick-rejection strategy rather than the egg-rejection strategy [39].

Broom et al. [2] used a model to examine the evolutionary reason for sequential defences, and the trade-off between the earlier and later defences. The model included the consideration of both "fitness-related investment" and "damage costs", and two levels of defences. The model shows that, when the investment costs are very high, and the benefits in investing in both defences are very low (the defences have low efficacy for protection, attacks are rare, and the exposure time of prey to predators is short), then the optimal choice is not to invest in either of the two defences. When the cost/benefit ratio of the first defence is much higher compared to the second defence, then the investment will be concentrated in the second defence; and vice versa for the other defence. When the ratio cost/benefit is similar and not very high for both defences, then the investment will be in both defences. Therefore, there is a trade-off between the first and second defence, and the evolution will choose the more effective defence over the less effective one. Also, if both defences are

similarly effective and not very costly, then evolution will choose both defences.

The investment in earlier and later defences might also be influenced by the environments that the victim inhabits. For example, the investment allocation of constitutive defences and induced defence in pines is found to be different in different environments [1]. Constitutive defences increase at higher latitudes and elevation, and colder temperature areas where growth rate decreases, whereas induced defences increase in the opposite environmental areas. Therefore, there is not only trade-off between the investment in earlier and later defences, and the investment ("fitness-related investment") in both defences are associated with the growth rates, but also some external factors, e.g. the living environment.

In the second chapter, I will use a mathematical model to explore the investment defence allocation strategy. I will answer the question of when organisms invest in multiple defences rather than single defences. I will also find out whether more will be invested in earlier or later defences. This model has two extensions, in comparison to Broom et al. [2]. First, the model will include n levels of defences rather than only two because some organisms in nature have more than two levels of sequential defences, so the model can also be applied to explore the defences investment allocation strategies in those organisms. Second, a general form rather than a linear form of "fitness-related investment" function will be used, since the form of function might influence the results.

The variances of sequential defences

One defence can be variable among individuals in the population. Speed et al. [40] showed that plants' chemical defences can be variable both in quantities of toxins and in the chemical constituents. Other non-chemical defences such as the length of thorns and camouflage colours can also be variable in plants. Defence behaviours in animals can also be variable. For example, the ability to run away from predators cannot be exactly the same between individuals, since there might be some differences in the physical structures in their bodies.

Although better defences are found to have better protective effects for victims, variations in defence still persist. Higher concentrations of glucosinolates and larger density of trichomes can reduce herbivore by beetles in *Arabidopsis thaliana* [41], but variations of both glucosinolates and trichomes are still found to be a heritable trait [42]. The question is:

why does variation persist and evolve across generations?

The term "mutation-selection balance" is sometimes used to describe the equilibrium when the rate at which the deleterious alleles are initiated by mutation equals the rate at which the deleterious alleles are eliminated by selection [43, 44, 45]. Similarly, the mutation-selection balance can also be applied to mutation-selection competition in defence phenotypes. The variance of defence phenotypes in the population will decrease when the selection force pushes the defence phenotypes towards the ideal phenotype for the organisms. At the same time, all of the phenotypes in the population have some chance to mutate to be different (more beneficial or more deleterious) in the offspring, so the variance increases. Therefore, both the force of selection that accumulates the phenotypes, together towards the ideal phenotype and the force of mutation that spreads the phenotypes away, determine the variation of the phenotypes. The variance reaches an equilibrium when both forces balance out.

In the third chapter of this thesis, the variance equilibria in the mutation-selection balance will be explored. Previous research about mutation-selection usually focuses on the equilibrium of the ratio between beneficial and deleterious alleles [43, 44, 45] or on the distribution in mutation-selection balance in discrete trait population (quasispecies model [46]), but the distribution (variances) in mutation-selection balance in a continuous trait population have rarely been explored. Using a mathematical model, I will explore the evolution of variances across generations, and how the evolution of variance reaches the mutation-selection balance (equilibrium).

In the sequential defence scenario, the mutation-selection balance may have different equilibrium value according to the position of the defence in the sequence. Studies in plant defences show that variations in earlier defences (physical, morphological defence) are better at predicting the damage by herbivores than later defence (toxic secondary metabolites, [47, 48]). It means that the earlier defences are less tolerant of the deviation from the most adaptive defence than the later defences. It also shows the possible imbalance in the variances in earlier and later defences. Using the same mathematical model, the relation between the variances in the earlier and later defences during evolution and in the equilibrium will also be explored in the third chapter.

1.3.3 Defences acting synergistically

Synergistically-acting Defences

Some defences can work synergistically together to achieve a better effect than if each worked independently. For example, the defence aposematism is usually considered to be synergistically working with chemical defences [12, 13, 14, 49]. The aposematic colours will attract the predators' attention, thereby making the victims more conspicuous (and possibly more dangerous) than cryptic colours. The chemical defences themselves have the effect of defence, but the aposematic colours constitute further visual signals of the unseen defences, that can help to warn predators to stay away without testing and damaging the victims. Also, aposematic colours are found to assist learning to avoid the defences [50]. So predator attack is reduced when victims can show aposematic colours as signals of their chemical defences.

The evolution of synergistically-acting defences

Synergistically-acting defences enhance the effect of each other. Gilman et al. [3] use a model to explore the reason for the evolutionary reason for this kind of multiple defences. The model explores the effect of both the number of defences and the correlation between traits in evolution. The model also includes the consideration of both "fitness-related investment" and "damage costs". It shows that the increasing number of defences and the strength of correlation between traits would both increase the probability that victims can survive from enemy attack. The number of defences matters because there is an unequal relationship between the victims and predator– the victims can survive only if one defence holds, however, all of the defences need to be breached for the enemies to succeed [3]. The synergistic act matters because it can strengthen the effect of each of the defences by the interaction and therefore increase escape rates.

The evolutionary order of two synergistically-acting defences

Since the evolution of new defences is usually instantaneous compared to the persistence of existing defences, the simultaneous evolution of two new defences is unlikely to happen.

Therefore an interesting question is which of the two synergistic defences evolves first. For example, the spikes on the shells of turtles (e.g. *Macrochelys temminckii*) are likely to be a later evolved defence on turtles' shell defence itself, because most turtles do not have spikes. Aposematic defences usually function as signals of chemical defences [12, 49, 13, 14]. Since the aposematic organisms are usually found also to have toxins, but the organisms with toxins can be cryptic, not aposematic species, it is usually assumed that chemical defences evolve earlier than the corresponding aposematic defences [14]. Also, the aposematic species without toxins are regarded to mimic some other aposematic organisms with toxin [51].

In comparison to the order in which aposematic and chemical defences evolved, the order in which aposematism and group-living evolved is less obvious. It could be the case either that solitary aposematic species evolved to live in groups, or that species that live in groups evolved aposematic colouration. This is a particularly interesting question as both the evolution of group living and aposematism increase the conspicuous effect of the other. Therefore, on the one hand, aposematism could evolve in the group-living species, due to kin selection [52, 53, 54, 55, 56];on the other hand, it is also possible that living in groups can enhance the effect of aposematism that shows the chemical defences in the organisms, so group-living evolves later than aposematism [57, 58, 59, 60, 61]. In the fourth chapter, this question is examined by exploring all of the possible pathways between combinations of colour traits (aposematism vs crypsis) and group traits (group-living and solitary-living) using phylogenetic comparative methods. The evolution from aposematism to crypsis and the evolution from group-living to solitary-living are also included in consideration as these possibilities cannot be excluded, although previous research assumes that evolution occurs in the opposite direction.

The aposematism & group-living defences and diversification in caterpillars

Defences can not only influence the survival of that population or species (microevolution) as mentioned above, they are also expected to have a positive influence on diversification rates, which means anet increase in the number of species within a certain lineage (macroevolution). The idea comes from Ehrlich and Raven's "escape and radiate" theory [62, 63]. The theory predicted that defences help the organisms enter into a new adaptive zone, which increases the chances to characterise new families.

Defences are predicted to increase the diversification rates in two ways. First, effective defences can help to protect the population. On the one hand, they increase the population size, so increasing the chances of mutation and recombination, which might help to generate new species [64]. On the other hand, an increased population size decreases the chance of the species' extinction. Both of these can increase diversification rates. Second, with an effective defence, the populations are able to live in either more variable ecological environments or in a wider range of living areas. So more interactions with the new and wider environment and the new capacity for populations' living can increase the chance of the formation of new species [65]. Finally, species diversity itself was found eventually to form the basis for new species [66].

Some studies have tried to explore the relationship between defences and diversification rates [15, 17, 18, 19, 20, 21, 67] but the evidence remains limited. Defences, such as aposematism, gained more consistent positive evidence [20, 21, 67] than some other defences, such as chemical defences, which produced some ambiguous results regarding their influence on the diversification rates (e.g. chemical defence might increase diversification rates [18, 62], decrease diversification rates [15, 67], or have no influence [21]). The fifth chapter is designed to test whether aposematic defences and group-living defences can influence the diversification rates in Macrolepidoptera caterpillars, which aims to bring some new evidence to the "escape and radiate" theory.

1.4 The outline and aims of the following thesis

This thesis will focus mainly on the connections between defences. Multiple defences are usually shown to respond towards the different circumstances (e.g. enemies, environments, living stages), that the victims face (section 1.3.1). However, research about the connections between defences is relatively limited (section 1.3.2, 1.3.3). In this thesis, the connection between earlier and later defences deployed sequentially, and the connection between two synergistically deployed defences will be mainly analysed. I will show the evolutionary reason for multiple defences, the investment trade-off among defences, the evolution of distribution variances across generations, the mutation-selection balance in multiple defences, the coevolution between different defences, and the relationship between defences and species diversification.

The second chapter uses mathematical models to explore the evolution of sequential defences. Compared to Broom et al [2], a general investment function rather than the linear investment functions is used. Since the forms of investment functions can influence the results (as it will be shown), the generalised function can let us get the results without worrying about whether the investment function is the same as what happens in nature, which we might never know. Also, I generalise the number of defences to n, compared to only two in Broom et al [2], as more than two levels of defences are frequently observed in reality. In the chapter, the evolutionary reason for multiple defences (why multiple defences are selected compared to only one "super-defence") and the trade-off between earlier and later defences will be studied.

In the third chapter, the evolution of variances of the earlier and later defences will be explored. Current literature about the variances of defences is quite limited, so the aim is to help fill this gap in the research. A mathematical model that considers both selection force and mutation force to show how the distribution of defence phenotypes in the earlier and later defences evolves across time will be developed. This chapter also compares the equilibrium variances of the distributions for earlier and later defences in the mutation-selection balance, and also see how different factors influence the variances.

In the fourth chapter, the dynamic coevolution of colour defence traits (aposematism and crypsis) and the group defence traits (group-living and solitary-living) in caterpillars are explored. Compared to the previous research, that is only focused on the evolution from crypsis to aposematism and from solitary-living to group-living, I also include the possibility of the reverse being the case. The dynamics of the combination of the two traits is a more comprehensive approach to studying the problem, and the results show a new understanding of the coevolution of the two traits.

In the fifth chapter, whether both defences (aposematism and group-living defences) can influence the diversification rates is explored. The results will add further evidence to the currently limited research about Ehrlich and Raven's "escape and radiate" theory [62, 63].

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Chapter 2

A theory for investment across defences triggered at different stages of a predator-prey encounter

2.1 Introduction

All organisms face threats from enemies, be they predators attacking animal prey, herbivores eating plant tissue, or pathogens and parasites feeding on host tissues. The coevolution between such enemies is a major driving force in evolution, which has contributed substantially to the diversification of defensive mechanisms deployed by organisms, and indeed of life's forms [1]. A major and important general biological question here is why organisms often invest in several defensive mechanisms, rather than putting all their defensive resources into one highly effective "superdefence". Why, for example, do most animals and plants not merely invest in toxins, but often invest additionally in physical and behavioural defences? One answer is that the components of multiple defence suites each target alternative types of enemy, in which case we could expect a positive association between the number of defences deployed and the number of different classes of enemy. A second answer is that multiple defences act simultaneously and perhaps synergistically,

so that a greater total level of protection is achieved per unit invested when an enemy is assaulted by e.g. physical and chemical defences together. Alternatively defences may act one after another, presenting predators with a sequence of barriers that enemies must cross to gain the resources presented to them by the victim. Here we focus on this third explanation, and consider the evolution of multiple, sequentially acting defences.

A good reason for assuming that many defences act sequentially - and hence the focus of this chapter - is that interactions between victims and enemies can often be split into a number of stages at which one or more defences can be deployed. Although a variety of different descriptions of this process have been suggested (see ref. [2] for a review), the most commonly used in the context of animal defence is that given by Endler [3] who splits the process up into six sequential stages: (i) spatial and temporal proximity of predator and prey, (ii) detection of prey by predator, (iii) identification of prey by predator, (iv) chase or stalking by the predator to close the distance to the prey, (v) subjugation of the prey, and (vi) final consumption. Attack by herbivores on plants can be similarly described in a sequence of stages, though here without the behaviour of chase by the predator.

Defensive traits extend across all phases of attacks. For example, prey can reduce the risks: of spatial and temporal proximity by avoiding habitats where predators are more common; of detection, through lack of movement and cryptic appearance; of identification, through mimicry or masquerade; of predators closing in, through fleeing; of subjugation, through struggle, spines or production of slippery secretions; and finally, prey can prevent the risk of consumption, through chemical toxins. Hence it is possible for prey to employ defences at all stages of the predation sequence in order to curtail attack.

Defences are often thought costly [2, 4] and investment in defence acting at one stage in the sequence might reduce the benefit of investment in defences that act at later stages. Hence, it seems logical that investment should be biased towards earlier stages, as was argued by Endler [3]. However, it is clear that in the natural world sometimes there is investment in later-acting defences. Here we ask whether there could be a general framework for understanding investment in defences that act in sequence (as highlighted by [2]). Our aim in this chapter is to introduce a simple but general theoretical description of a combination of defences acting at different stages in the predatory sequence in order to make predictions about how prey should best allocate investment across different defensive stages. The model can, in our view, provide a flexible and predictive framework for understanding strategies

of investment in multiply defensive systems in many biological contexts, including animal prey. We also explore its application to the evolution of sequentially acting plant defences, proposing a new explanation for the otherwise puzzling lack of effects on herbivory for variation in plant chemical defences [5].

2.2 The Sequential Defences Model

We assume that the prey can invest in at most n stages of defence, which the predator experiences sequentially. We denote each defence stage by the order i ($i = 1, \ldots, n$) in which it is encountered (so $i = 1$ is the first defence encountered, and $i = n$ the last). We define s_i ($0 \leq s_i < 1$) as the success probability of the prey's i-th defence, i.e. the probability that, if the predator reaches defence i, then it fails to overcome that defence. The effectiveness of each defence depends upon the level of investment in it. We define I_i as the (non-negative) investment made in defense i, so

$$I_i = I_i(s_i) \tag{2.1}$$

is a non-decreasing function ($I_i'(.) \geq 0$), so a defence with a higher probability of success requires higher investment by the prey. We also assume that, if the prey invests nothing in a defence, then the success probability of that defence will be 0: $I_i(0) = 0$. It would arguably be more natural to consider the investments I_i as the fundamental variables of the model, and survival s_i as being a function of I_i, but our approach is formally identical (provided $I_i(\cdot)$ is monotonic, so there is a 1-to-1 relationship between I_i and s_i) and turns out to be more convenient to analyse.

Note that while predation pressure does not explicitly appear in the model, it is present implicitly because it affects the survival probabilities (or, more precisely, the relationship between I and s). The optimal strategy might be quite different among different populations, facing different environments and predation pressures. We are interested in the evolutionary defence strategy for a certain population. It is quite often observed that many individuals in a certain colony have the similar kind of defence strategies (e.g. similar level of aposematism or camouflage). We think of this as the optimised defence strategy averaged across generations and across populations. The form of the model is consistent with a single attack, but could also be though to represent a number of attacks. The latter are particularly appropriate for plants mounting defences against herbivores, where there

could be many attackers, each of which only does a small amount of damage. In that case we still can still think of defences being "breached" with a certain probability, even though there are many individual attack events. Here, predation pressure affects the probability that a defence is breached as well as the "tested costs" (because it affects the average number of times a defence is tested).

We assume that the prey has a total amount of resource, I_T, available for all defences. We define I_A as the investment in all the defences, so

$$I_A = \sum_{i=1}^{n} I_i(s_i) \leq I_T \tag{2.2}$$

We define $C(I_A)$ as the fitness cost of making investments across the various defences, in whatever division, so that this total investment amount is I_A. We assume that $C(0) = 0$ (when there is no investment in defence, the investment cost is zero). The residual amount of resources left after investment across all the defences, $I_T - I_A$, can effectively then be used as additional investment in non-defensive fitness-enhancing activities. Thus we assume $C(.)$ is an increasing function of I_A (i.e. $C'(.) > 0$).

We further assume that, if defence i is tested by the predator, then (even if the defence holds) there is a cost c_i (≥ 0), henceforth referred to as "tested cost", that can be considered as the injury risk of being exposed to the predators after defence $i-1$ is breached. (Note that c_i can be 0, which means that the tested cost is zero; e.g the tested cost of crypsis in a nocturnal moth might be zero.) In this assumption, since there is no defence before the first defence, we think that the first defence is always exposed and tested by the predators (although the tested cost for the first defence can be zero, $c_1 = 0$). Alternatively, c_i can be thought of as the costs incurred when a predator triggers the defences at stage i. These need not be solely risk of injury, but might additionally or alternatively be time, energy or other resource spent in the deployment of the defence. Like the model from Wilkening [6] discussing layered defences in military use, we calculate the probability that each defence is tested and holds (i.e. is not breached); and we also calculate the corresponding fitness when that defence is tested and holds. Multiplying them together, we get the expected fitness contribution from the eventuality where that defence is tested and holds. The overall fitness which we care about is the sum of all these terms. Here, fitness means the average number of viable offspring that an individual produces, and by assuming that this number

decreases as successive defences are tested and/or breached we are able to represent many different possible reproductive life histories (continuous reproduction, semelparity, etc.). We consider two particular scenarios: (A) the prey has positive residual fitness (e.g. still alive and can reproduce) when all defences are breached; (B) the prey has zero residual fitness (e.g. dies before reproducing) when all defences are breached. We wish to find the defence strategy that maximises R.

For scenario (A), when the prey still has positive residual fitness when all the defences are breached, the expression of the overall fitness R of the prey for a given investment strategy $S = (s_1, s_2, \ldots, s_n)$ is as follows.

$$R(s_1, s_2, \ldots, s_n)$$

$$= s_1(1 - C(I_A) - c_1)$$

(the fitness when the first defence is tested, but not breached)

$$+(1 - s_1)s_2(1 - C(I_A) - c_1 - c_2)$$

(the fitness when the second defence is tested, but not breached)

$$+...$$

$$+(1 - s_1)(1 - s_2)...(1 - s_{n-1})s_n(1 - C(I_A) - c_1 - c_2 - ... - c_n)$$

(the fitness when the $(n-1)$th defence is tested, but not breached)

$$+(1 - s_1)(1 - s_2)...(1 - s_n) \cdot (1 - C(I_A) - c_1 - c_2 - ... - c_n)$$

(the fitness when all the defences are breached)

$$= 1 - C(\sum_{i=1}^{n} I_i(s_i)) - c_1 - \sum_{j=2}^{n} c_j \prod_{k=1}^{j-1}(1 - s_k) \tag{2.3}$$

Note that we have assumed that the fitness when all the defences are breached is the same as the fitness when the $(n-1)$th defence is tested, but not breached, since no further tested costs are incurred after the nth defence is breached.

In many cases in the real world, the prey dies or effectively dies with 0 fitness left to reproduce when all the defences are breached. We therefore consider an alternative scenario (B) where the fitness when all the defences are breached is 0 instead of $(1 - C(I_A) - c_1 - c_2 - ... - c_n)$. Then the fitness function is as follows.

$$R(s_1, s_2, \ldots, s_n) = (1 - \prod_{j=1}^{n}(1 - s_j))\Big(1 - C(\sum_{i=1}^{n} I_i(s_i))\Big) - c_1 - \sum_{j=2}^{n} c_j \prod_{k=1}^{j-1}(1 - s_k) + (\sum_{j=1}^{n} c_j)\prod_{j=1}^{n}(1 - s_j) \tag{2.4}$$

These two scenarios represent the two extreme possibilities for the fitness that ensues when all defences are breached: fitness is not decreased further by the nth defence failing in scenario (A), whereas all fitness is lost in scenario (B) if the nth defence fails. We expect that the results for an intermediate scenario will lie between those for these two extreme scenarios.

If the organism invests less than the maximum available resources in defences, then those resources are available for reproduction and other fitness-enhancing activities. This is represented in the model by the term $-C(\sum_{i=1}^{n} I_i(s_i))$ in both equations (2.3) and (2.4)), which tends to increase fitness if $I_A = \sum_{i=1}^{n} I_i(s_i)$ is decreased. However, due to the other terms in s_i it is not clear without analysis whether I_A is less than or equal to I_T in the optimal strategy.

2.2.1 When testing defences are costly, later defences receive lower investment

If the investment function is the same for all defences, $I_i(\cdot) = I(\cdot)$, we can show that the optimal solution $S = (s_1, s_2, ..., s_n)$ maximising the fitness function R in (2.3) and (2.4) always satisfies the following relation when the tested costs c_i are strictly positive $(c_i > 0 \, \forall i)$.

$$s_1 \geq s_2 \geq ... \geq s_n. \tag{2.5}$$

This is because, for any i such that $s_i < s_{i+1}$, we can always make R larger by switching

the value of s_i and s_{i+1}, which will only change the term $-c_{i+1}\prod_{k=1}^{i}(1-s_k)$ (in the term $-\sum_{j=2}^{n} c_j \prod_{k=1}^{j-1}(1-s_k)$ in R) to $-c_{i+1}\prod_{k=1}^{i-1}(1-s_k)\cdot(1-s_{i+1})$ (larger than $-c_{i+1}\prod_{k=1}^{i}(1-s_k)$), with the other terms in R unchanged.

Since the investment function $I(s_i)$ is increasing, the relation that $s_1 \geq s_2 \geq ... \geq s_n$ means that

$$I(s_1) \geq I(s_2) \geq ... \geq I(s_n). \tag{2.6}$$

This shows that investment in earlier defences should never be less than than investment in later defences.

Note that, if $c_{i+1} = 0$, the above argument does not show that $s_i \geq s_{i+1}$, but rather than the fitness R is unchanged by switching the values of s_i and s_{i+1}. This means that, when one of the tested costs is zero, either (i) there is a unique optimal strategy, where $s_i = s_{i+1}$; or (ii) the optimal strategy is not unique, but the optimal strategy in which $s_j \geq s_{j+1}$, for all j has equal fitness to the best strategy where $s_{j+1} > s_j$ for some j. In any biologically realistic situation there will always be a cost — however small — to having a defence tested, but this case is still interesting because it shows what might evolve when the tested costs are very small.

2.2.2 Investing in multiple defences or in a single defence?

The best strategy for the organism might be to invest in multiple defences, with (according to the above result) higher investment in earlier than later defences. On the other hand, the best strategy might be to invest in a single defence, which the above argument shows should be the first one. As we will see later, either of these outcomes can occur, depending on the details of the investment function I. To show this, first we find conditions that the optimal solution must satisfy. To find the maximised R constrained by variable boundaries $0 \leq s_i < 1$, and resource boundary $\sum_{i=1}^{n} I(s_i) \leq I_T$, we write a Lagrange function for the overall fitness function (2.3) and (2.4).

$$L(s_1, s_2, \ldots, s_n; \lambda_1, \lambda_2, \ldots, \lambda_n) = R(s_1, s_2, \ldots, s_n) + \sum_{i=1}^{n} \lambda_i(1 - s_i) + h(I_T - \sum_{i=1}^{n} I(s_i)) \tag{2.7}$$

The necessary condition to get the maximised value R is given by the Karush-Kuhn-Tucker (KKT) condition coming from the above Lagrange function,

$$\frac{\partial L}{\partial s_i} = \frac{\partial R}{\partial s_i} - \lambda_i - hI'(s_i) \leq 0, \quad s_i \geq 0, \quad s_i \frac{\partial L}{\partial s_i} = 0 \qquad i = 1, ..., n \tag{2.8}$$

$$\frac{\partial L}{\partial \lambda_i} = 1 - s_i \geq 0, \quad \lambda_i \geq 0, \quad \lambda_i \frac{\partial L}{\partial \lambda_i} = 0 \quad i = 1, ..., n \tag{2.9}$$

$$\frac{\partial L}{\partial h} = I_T - \sum_{i=1}^{n} I(s_i) \geq 0, \quad h \geq 0, \quad h \frac{\partial L}{\partial h} = 0 \quad i = 1, ..., n \tag{2.10}$$

The second necessary condition (2.9) combined with $1 - s_i > 0$, is equivalent to

$$\lambda_i = 0 \tag{2.11}$$

The first necessary condition (2.8) is equivalent to

$$\frac{\partial L}{\partial s_i} = \frac{\partial R}{\partial s_i} - hI'(s_i) \leq 0, \quad s_i \geq 0, \quad s_i \frac{\partial L}{\partial s_i} = 0 \qquad i = 1, ..., n \tag{2.12}$$

(a)When $s_i > 0$: we have that $\frac{\partial L}{\partial s_i} = \frac{\partial R}{\partial s_i} - hI'(s_i) = 0$.

(b)When $s_i = 0$: we have that $\frac{\partial L}{\partial s_i} = \frac{\partial R}{\partial s_i} - hI'(s_i) \leq 0$.

For the third necessary condition (2.10),

(a)When $I_T - \sum_{i=1}^{n} I(s_i) > 0$: we have that $\frac{\partial L}{\partial h} = I_T - \sum_{i=1}^{n} I(s_i) > 0$, so $h = 0$ since $h\frac{\partial L}{\partial h} = 0$.

(b)When $I_T - \sum_{i=1}^{n} I(s_i) = 0$: we have that $\frac{\partial L}{\partial h} = I_T - \sum_{i=1}^{n} I(s_i) = 0$; so we still have $h \geq 0$.

To put them together, the necessary condition is equivalent to the following:

(I) When $0 < s_i < 1$,

$$\frac{\partial R}{\partial s_i} - hI'(s_i) = 0, \qquad h \geq 0, \tag{2.13}$$

$$\text{(and } h = 0, \text{ when inside the resource boundary } I_T - \sum_{i=1}^{n} I(s_i) > 0)$$

(II) When $s_i = 0$,

$$\frac{\partial R}{\partial s_i} - hI'(s_i) \leq 0, \qquad h \geq 0 \tag{2.14}$$

$$(h = 0, \text{ when inside the resource boundary } I_T - \sum_{i=1}^{n} I(s_i) > 0)$$

So far, the analysis has been the same whether we assume that the fitness after all defences are breached is zero (Scenario (B), equation (2.4)) or not (Scenario (A), equation (2.3)). For the following calculation, we assume scenario (B) only; the calculation for scenario (A) follows along similar lines, and has the same conclusion, and is presented in Appendix 2.6.2. Given the fitness function R in (2.4), the necessary condition for R is as follows,

(I) When $0 < s_i < 1$,

$$\begin{aligned}
&\frac{\partial R}{\partial s_i} - hI'(s_i) \\
&= \frac{1}{1-s_i}\prod_{j=1}^{n}(1-s_j)\big(1 - C(I_A) - \sum_{j=1}^{n} c_j\big) - (1 - \prod_{j=1}^{n}(1-s_j))\frac{\partial C(I_A)}{\partial s_i} + \frac{1}{1-s_i}\sum_{j=i+1}^{n} c_j \prod_{k=1}^{j-1}(1-s_k) \\
&- hI'(s_i) \\
&= 0 \quad (\text{ with } h \geq 0)
\end{aligned} \tag{2.15}$$

(II) When $s_i = 0$,

$$\begin{aligned}
&\frac{\partial R}{\partial s_i} - hI'(s_i) \\
&= \frac{1}{1-s_i}\prod_{j=1}^{n}(1-s_j)\big(1-C(I_A)-\sum_{j=1}^{n}c_j\big) - (1-\prod_{j=1}^{n}(1-s_j))\frac{\partial C(I_A)}{\partial s_i} + \frac{1}{1-s_i}\sum_{j=i+1}^{n}c_j\prod_{k=1}^{j-1}(1-s_k) \\
&- hI'(s_i) \\
&\leq 0 \quad (\text{ with } h \geq 0)
\end{aligned} \tag{2.16}$$

Now we have the necessary condition to maximise R— (2.15) and (2.16). Next, we are going to explore whether investment can happen in multiple defences or only in one defence.

Since the investment functions are the same for all the defences ($I_i(\cdot) = I(\cdot)$), we have that investment in earlier defences is always larger than investment in later defences (equation (2.5), (2.6)), so for some $j(< n)$,

$$1 > s_1 \geq s_2 \geq \ldots \geq s_j > s_{j+1} = \ldots = s_n = 0, \tag{2.17}$$

or

$$1 > s_1 \geq s_2 \geq \ldots \geq s_{n-1} \geq s_n > 0. \tag{2.18}$$

Note that when $j = 1$,

$$1 > s_1 > s_2 = \ldots = s_n = 0, \tag{2.19}$$

then the investment is concentrated only in the first defence.

We will now find the conditions that determine whether investment is concentrated only in the first defence, or in multiple defences.

Let us first assume that there are multiple defences ($2 \leq j \leq n$), then for some $i \in \{1, 2, ..., j-1\}$, we will have $s_i \geq s_{i+1} > 0$. Then from equation (2.15), we have (2.20) and (2.21).

$$\begin{aligned}
&\frac{\partial R}{\partial s_i}\cdot(1-s_i)-h(1-s_i)I'(s_i)\\
&=\prod_{j=1}^{n}(1-s_j)\big(1-C(I_A)-\sum_{j=1}^{n}c_j\big)-(1-s_i)(1-\prod_{j=1}^{n}(1-s_j))\frac{\partial C(I_A)}{\partial s_i}+\sum_{j=i+1}^{n}c_j\prod_{k=1}^{j-1}(1-s_k)\\
&-h(1-s_i)I'(s_i)\\
&=0, \qquad (2.20)
\end{aligned}$$

$$\begin{aligned}
&\frac{\partial R}{\partial s_{i+1}}\cdot(1-s_{i+1})-h(1-s_{i+1})I'(s_{i+1})\\
&=\prod_{j=1}^{n}(1-s_j)\big(1-C(I_A)-\sum_{j=1}^{n}c_j\big)-(1-s_{i+1})(1-\prod_{j=1}^{n}(1-s_j))\frac{\partial C(I_A)}{\partial s_{i+1}}+\sum_{j=i+2}^{n}c_j\prod_{k=1}^{j-1}(1-s_k)\\
&-h(1-s_{i+1})I'(s_{i+1})\\
&=0 \qquad (2.21)
\end{aligned}$$

The term $\prod_{j=1}^{n}(1-s_j)\big(1-C(I_A)-\sum_{j=1}^{n}c_j\big)$ in both (2.20) and (2.21) is the same, so that we have

$$\begin{aligned}
&-(1-s_{i+1})(1-\prod_{j=1}^{n}(1-s_j))\frac{\partial C(I_A)}{\partial s_{i+1}}+\sum_{j=i+2}^{n}c_j\prod_{k=1}^{j-1}(1-s_k)-h(1-s_{i+1})I'(s_{i+1})\\
&=-(1-s_i)(1-\prod_{j=1}^{n}(1-s_j))\frac{\partial C(I_A)}{\partial s_i}+\sum_{j=i+1}^{n}c_j\prod_{k=1}^{j-1}(1-s_k)-h(1-s_i)I'(s_i). \qquad (2.22)
\end{aligned}$$

Since $I_A=\sum_{i=1}^{n}I(s_i)$, the above is equivalent to

$$\begin{aligned}
&-(1-s_{i+1})(1-\prod_{j=1}^{n}(1-s_j))C'(I_A)I'(s_{i+1})-h(1-s_{i+1})I'(s_{i+1})\\
&=-(1-s_i)(1-\prod_{j=1}^{n}(1-s_j))C'(I_A)I'(s_i)+c_{i+1}\prod_{k=1}^{i}(1-s_k)-h(1-s_i)I'(s_i). \qquad (2.23)
\end{aligned}$$

$\Rightarrow$

$$-(1-s_{i+1})I'(s_{i+1})\Big((1-\prod_{j=1}^{n}(1-s_j))C'(I_A)+h\Big) = -(1-s_i)I'(s_i)\Big((1-\prod_{j=1}^{n}(1-s_j))C'(I_A)+h\Big)$$
$$+c_{i+1}\prod_{k=1}^{i}(1-s_k). \tag{2.24}$$

Since $C'(I_A)>0$ and also $1-\prod_{j=1}^{n}(1-s_j)>0$ and $h\geq 0$, we have that $\Big((1-\prod_{j=1}^{n}(1-s_j))C'(I_A)+h\Big)>0$, so that equation (2.24) is equivalent to

$$-(1-s_{i+1})I'(s_{i+1}) = -(1-s_i)I'(s_i) + c_{i+1}\frac{\prod_{k=1}^{i}(1-s_k)}{\Big((1-\prod_{j=1}^{n}(1-s_j))C'(I_A)+h\Big)}. \tag{2.25}$$

The last term in the right-hand side $c_{i+1}\frac{\prod_{k=1}^{i}(1-s_k)}{\Big((1-\prod_{j=1}^{n}(1-s_j))C'(I_A)+h\Big)}$ is positive when $c_{i+1}>0$, therefore

$$-(1-s_{i+1})I'(s_{i+1}) > -(1-s_i)I'(s_i). \tag{2.26}$$

which is the same to,

$$(1-s_{i+1})I'(s_{i+1}) < (1-s_i)I'(s_i). \tag{2.27}$$

The analyses for the fitness function (2.3) (in Appendix 2.6.2) are similar to the analysis for the fitness function (2.4) (from equation 2.15 to 2.27). As the relation between s_i and s_{i+1} for the fitness function (2.3) (equation B.11) is the same as the relation (2.27) for the fitness function (2.4), the following analyses hold for both (2.3) and (2.4).

If $(1-s)I'(s)$ is a monotonic decreasing function of s, (2.27) is inconsistent with $s_i \geq s_{i+1} > 0$, so we conclude that $s_{i+1}=0$. That is, investment can not be in multiple defences but only in the first defence (example see in Figure 2.3),

$$1 > s_1 > s_2 = \ldots = s_n = 0. \tag{2.28}$$

However, multiple defence can occur when the function $(1-s)I'(s)$ is an increasing function, at least for some range of values of s, in which case $s_i \geq s_{i+1} > 0$ ($i \in \{1, 2, ..., j-1\}$) is consistent with (2.27) (we give examples in Figure 2.1). Note that multiple defences are impossible if $(1-s)I'(s)$ is a decreasing function, but that $(1-s)I'(s)$ being an increasing function does not guarantee that the optimal solution has investment in multiple defences (see Figure 2.1).

Note that, if $c_{i+1} = 0$, as mentioned before in the section "When testing defences are costly, later defences receive lower investment", the optimal solution either (i) has the relation $s_i = s_{i+1}$ or (ii) is not unique, with one optimal solution having $s_i > s_{i+1}$ and the other being obtained by swapping the values of s_i and s_{i+1}. When the function $(1-s)I'(s)$ is an increasing function, we can prove that only $s_i = s_{i+1}$ occurs (Appendix 2.6.1 (i); for an example see in Figure 2.2). Similarly, when $c_{i+1} = c_{i+2} = 0$, the optimal solution will have the relation that $s_i = s_{i+1} = s_{i+2}$. In biologically realistic situations, tested costs will usually be nonzero, so since the fitness function R is continuous in c_{i+1}, we will have s_i being slightly larger than s_{i+1}.

When the function $(1-s)I'(s)$ is a decreasing function, we can prove that only $c_2 = 0$ changes the relation (2.28), and that $s_i = s_{i+1}$ is not possible in the optimal solution, and that the same amount investment will be concentrated only in the first or only in the second defence ($1 > s_1 > s_2 = s_3 = s_4 = ... = s_n = 0$ or $1 > s_2 > s_1 = s_3 = s_4 = ... = s_n = 0$) (Appendix 2.6.1 (ii)(iii)). Similarly, when $c_2 = c_3 = 0$, investment will only be in one of the first three defences. However, a small tested cost will drive the investment to be only in the first defence (For example see in Figure 2.3, 2.4).

2.3 Examples of investment in defences

We will give numerical examples for the cases when (1) investment happens in multiple defences, (2) only in one defences, and also (3) the investment functions are different, so that the investment in earlier defences can be either higher or lower than in later defences. The investment functions for all the three cases are given in the examples below. To show a numerical result of the optimal defence strategy, we further specify the expression for the cost function $C(I_A)$ as follows,

$$C(I_A) = {I_A}^a, \quad a \geq 1 \tag{2.29}$$

We assume that $a \geq 1$, since we expect that the marginal investment cost in defences is non-decreasing in respect to the defence investment (no less additional investment cost for additional amount of investment when the total amount of investment becomes larger). For simplicity, we consider scenario (B) (where the fitness is zero if all defences are breached), and assume that I_T is large enough that, in the optimal strategy, the organism does not need to invest all of its resources in defences ($I_A < I_T$), so that we do not need to consider I_T when maximising the fitness function (2.4).

We use a heuristic search algorithm to find the optimal investment strategy. The search starts at an initial point $S_0 = (s_{10}, s_{20}, s_{30}, s_{40})$. First, we calculate the value of R at this point, and then search whether there exists higher value of R in the positive direction of the first axis, through calculating the value of R at $(s_{10}+\delta_0, s_{20}, s_{30}, s_{40})$, where δ_0 is initial search step. If the value is higher, then we double the search step value and and do the search again, and repeat it until we find the maximum value of R and the corresponding value $S_1 = (s_{11}, s_{20}, s_{30}, s_{40})$; If however the value is not higher, we do the same procedure in the negative direction of the first axis to find the the maximum value of R and the corresponding value $(s_{11}, s_{20}, s_{30}, s_{40})$. We then do the same process in all the axes, and after that we get the corresponding value $S_1 = (s_{11}, s_{21}, s_{31}, s_{41})$. Second, we do the same as the first to find $S_2 = (s_{12}, s_{22}, s_{32}, s_{42})$ except that we shorten the initial search step to be $\frac{\delta}{u}$ (where $u > 1$). Third, we let the initial search step to be $\frac{\delta}{u^2}$ and do the same. We repeat this process until (e.g. at the n-th time, we find $S_n = (s_{1n}, s_{2n}, s_{3n}, s_{4n})$) the initial search step is less than a threshold ϵ_1 and the distance between the last two corresponding points $d(S_{n-1}, S_n)$ is less than a threshold ϵ_2, then stop.

Note that the above process might only find a local, rather than global, maximum. To solve this problem, we divide each of the interval $(0, 1]$ (note that each probability $s_i \in (0, 1]$) in each axis into m equal subintervals, and since we have four levels of defences, altogether, we have m^4 subareas. Then we do the same process as above to find all the m^4 local maxima. Theoretically, if m were large enough, we would have the global maximum in one of our searched results, and the largest local maximum is the global maximum. Due to computational limitations, we only devide into $3^4 = 81$ subareas. However, in all cases we found that, the 80 out of 81 subregions did not hold the largest local maximum, because those local maxima were on the boundaries of the subregions. This suggests that R does not have multiple stationary values, and that the largest local maximum we found is indeed the global maximum.

In our example specifically, the initial start point S_0 is given by $s_{1i} = 0.3, 0.6, 0.9$ in the three subintervals respectively ($i = 1, 2, 3, 4$) ($3^4 = 81$ start points for the 81 subareas in total), and $\delta_0 = 0.35$, $u = 1.5$, $\epsilon_1 = \epsilon_2 = 0.0001$.

2.3.1 Example of investment in multiple defences

As described above, when $(1 - s)I'(s)$ is an increasing function, investment could happen in multiple defences. As an example for this relation, we use the investment function of the form

$$I(s) = k(\frac{1}{1-s} - 1)^b, \quad i = 1, ..., n, \qquad k > 0, b > 1. \tag{2.30}$$

For this function, $(1 - s)I'(s) = kb(\frac{1}{1-s} - 1)^{b-1}\frac{1}{1-s}$, which can easily be shown to be an increasing function, when $k > 0$, $b > 1$.

Specifically we let $a = 2$, $b = 2$, $k = 0.2$, and the total number of defences be four.

Figure 2.1 gives that the optimal investment is concentrated in the only the first defence/ the first two defences/ the first three defences/ in all the four defences.

Figure 2.2 gives that for a specific i ($i = 1, 2..., N - 1$), if $c_{i+1} = 0$, then the investments in the i-th and $(i + 1)$-th defence are the same ($s_i = s_{i+1}$).

2.3.2 Example of investment only in the first defence

As described above, if $(1 - s)I'(s)$ is a decreasing function, then investment only happen in the first defence (when $c_2 > 0$). As an example for this relation, we use the investment function

$$I(s) = -k(\ln(1 - s) + ds \qquad k > 0, d > 0. \tag{2.31}$$

For this function, $(1 - s)I'(s) = k + d(1 - s)$, which is a decreasing function. Then we set the parameter values $a = 2$ to do the simulations as in the above example. Figure 2.3 shows that the optimal investment is concentrated only in the first defence. Figure 2.4 shows that, when $c_2 = 0$, there are two optimal solutions with one optimal solution

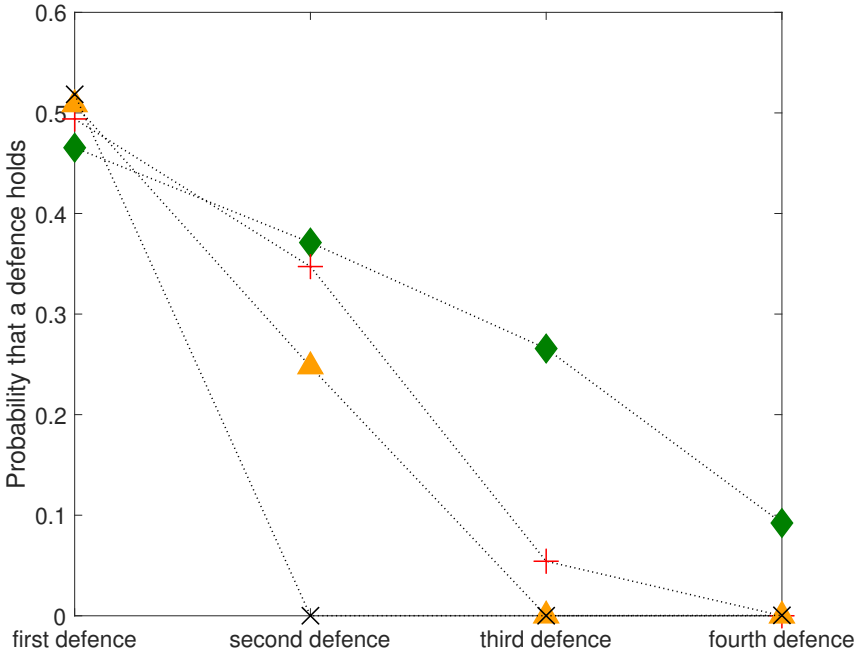

Figure 2.1: For investment functions I where $(1-s)I'(s)$ is an increasing function, prey can invest in multiple defences but always invest more in earlier defences. Here, the investment function is given in (2.30) and the cost function is given in (2.29). The vertical axis is s, the probability that a defence is not breached when tested, and is zero when investment in that defence is zero. Depending on the tested costs, the prey can invest in: all defences ($c_i = 0.2\forall i$, green rhombus); the first three defences only ($c_i = 0.3\forall i$, red cross); the first two defences only ($c_i = 0.4\forall i$, yellow triangle); or only the first defence ($c_i = 0.5\forall i$, black cross). Other parameter values: number of defences=4; $a = 2$, $b = 2$, $k = 0.2$.

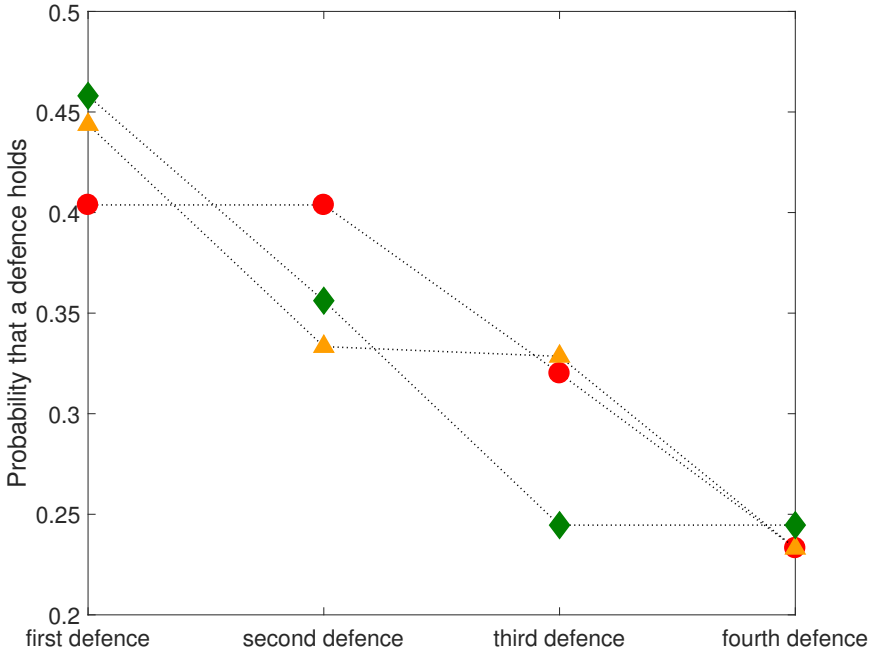

Figure 2.2: For investment functions I where $(1-s)I'(s)$ is an increasing function, prey can invest the same amount in two successive defences if the later defence has tested cost zero. Here, the investment function is given in (2.30) and the cost function is given in (2.29), and the tested costs are $c_i = 0.2$ for all values of i except one. Optimal strategy is to invest the same in: first and second defences when $c_2 = 0$ (red circles); second and third defences when $c_3 = 0$ (orange triangles); third and fourth defences when $c_4 = 0$ (green diamonds). Other parameters: number of defences=4; $a = 2$, $b = 2$, $k = 0.2$.

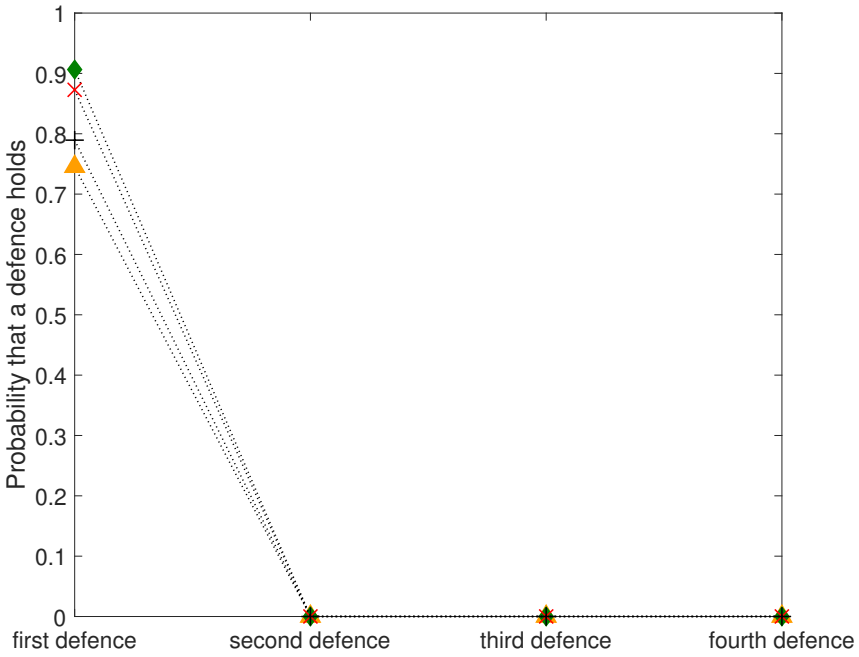

Figure 2.3: For investment functions I where $(1-s)I'(s)$ is a decreasing function, the optimal strategy is to invest in one defence only; this will be the first defence when the second defence has nonzero tested cost, $c_2 \neq 0$. Here, the investment function is given in (2.31), the cost function is given in (2.29), and the tested costs are $c_i = 0.2 \forall i$. Different symbols correspond to different values of parameters d and k: $(d,k) = (0.1, 0.1)$ (green rhombus); $(d,k) = (0.2, 0.1)$ (red cross); $(d,k) = (0.1, 0.2)$ (black cross); $(d,k) = (0.2, 0.2)$ (yellow triangle). Other parameters: number of defences=4; $a = 2$.

having $s_1 > s_2 = s_3 = s_4 = 0$ (investment concenrated only in the first defence), and the other being the swapped values of s_1 and s_2 (investment concenrated only in the second defence).

2.3.3 Example of different investment functions among defences

When the investment functions are different among defences (e.g. the value of k might be different in the investment function (2.30), which correponding to the efficiency to make

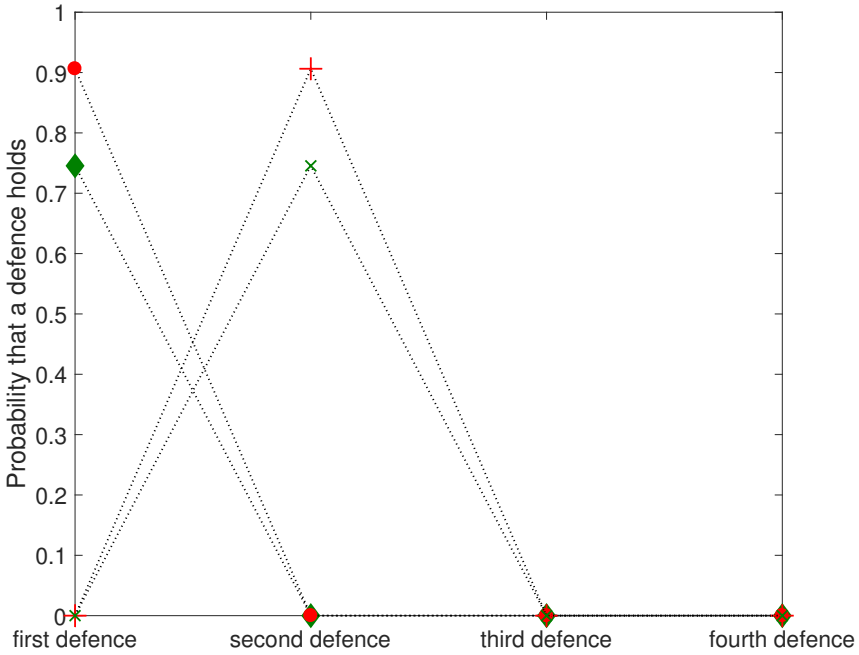

Figure 2.4: For investment functions I where $(1-s)I'(s)$ is a decreasing function, the optimal strategy is to invest in one defence only, but is degenerate when the second defence has tested cost zero ($c_2 = 0$): the fitness is the same whether the prey invests in the first defence only, or invests the same resources in the second defence only. Here, the investment function is given in (2.31), the cost function is given in (2.29), and tested costs are $c_2 = 0$, $c_i = 0.2$ for $i \neq 2$. Different colours correspond to different different values of parameters d and k: $d = k = 0.1$ (red); $d = k = 0.2$ (green). Different symbols distinguish the two optimal solutions: investment in first defence (red circle and green diamond); investment in second defence only (red cross and green cross). Other parameter values: number of defences=4, $a = 2$.

resources into defences might be different among defences),

$$I_i(s) = k_i(\frac{1}{1-s} - 1)^b, \quad i = 1, ..., n, \qquad k > 0, b > 1, \tag{2.32}$$

it is possible that investment in the later defences are higher than in the earlier defences. When the investment is more efficient to make resources into defences, in the later defences than in the earlier defences ($k_i > k_{i+1}$), the investment in later defences might or might not be higher than in the earlier defences (see the black cross or the red cross in Figure 2.5). However, when the investment in earlier defences is more efficient or equally efficient to make resources into defences than in the later defences ($k_i < k_{i+1}$), investment will be higher in the earlier than in the later defences (see the yellow triangle or the green rhombus in Figure 2.5).

2.4 Discussion

Endler [3] argued that prey should generally invest preferentially in defences that act early in the predation sequence, in part because defences met earlier in a sequence will on average be deployed more frequently and in part because he expects late acting defences to be less efficient (higher k_i values in (2.32)). We have shown however that the skew will occur when the investment function is the same for all the defences (so the efficiency k_i is equal across defences) provided there is a risk-of-injury (c_i) and other cost ($C(I_A)$) associated with implementing each in a set of sequentially organised defences. Also, we found that under some conditions defence investment will concentrated only in the first defence, while, under other conditions, investment can be distributed in several defences with more investment in earlier than in later defences. We suspect such costs will be common. We also suspect that Endler's assumption [3] that later-acting defences will be inherently more expensive for a given level of effectiveness (k_i increases with i) might hold generally (though it needs to be demonstrated), and this would more likely to further exaggerate the skew towards earlier-acting defences (yellow triangle in Figure 2.5). However, if the effectiveness of later defences is much higher, investment in later defences could be higher than in earlier defences. Our general theory and predictions allow us to synthesize previous more system-specific work on multi-component defences, and we now consider its application in specific biological and theoretical contexts, starting with plant defence against insect herbivores.

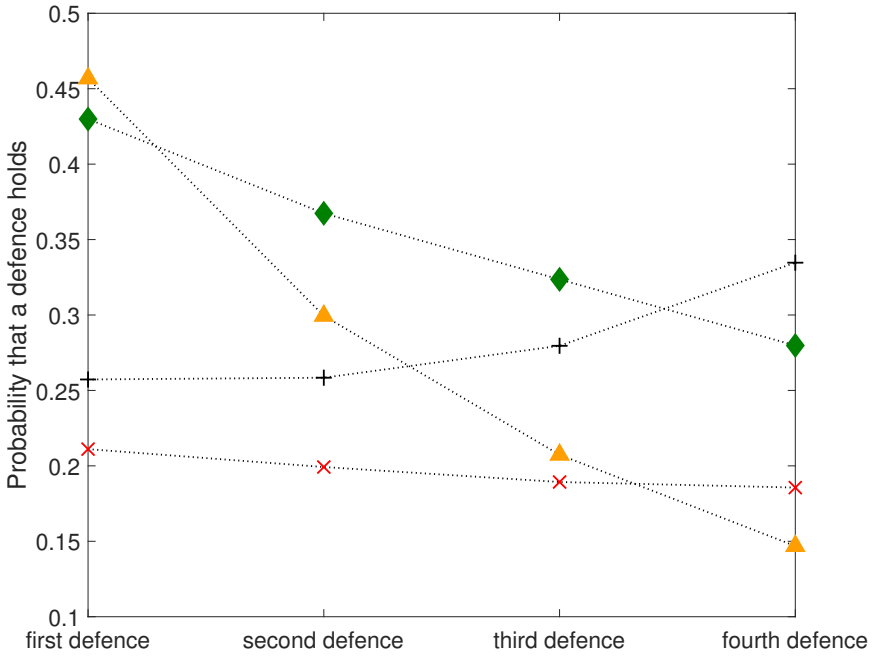

Figure 2.5: The optimum strategy might be to invest more in later defences, if the different defences do not have the same investment functions. Here, the investment function is given in (2.30) and the cost function is given in (2.29). The prey should invest more in earlier defences when defences have the same cost functions ($k_i = 0.2 \forall i$, green rhombus) or when later defences are more costly ($(k_1, k_2, k_3, k_4) = (0.2, 0.4, 0.6, 0.8)$, yellow triangle). However, when later defences are less costly, the optimal strategy might be to invest more in earlier defences ($(k_1, k_2, k_3, k_4) = (1.6, 1.4, 1.2, 1.0)$, red cross) or more in later defences ($(k_1, k_2, k_3, k_4) = (0.8, 0.6, 0.4, 0.2)$, black cross). Other parameters: number of defences=4; $a = 2$, $b = 2$, $c_i = 0.2 \forall i$.

2.4.1 Application to Plant Defences

It is common, when a victim is an animal prey, that it is killed and eaten (so has zero fitness, if it has not already reproduced) if the predator overcomes all of its defences (scenario (B)). Our model can also apply to many plant-herbivore interactions in which a small insect damages, but does not kill, the plant on which it is feeding (Speed *et al.* [7] also described a related model for these). In scenario (A), breaching the final defence does not cause further fitness cost on the prey, so the fitness keeps the same between when the final defence is tested but not breached and when the final defence is breached. We have done the analyses for both this extreme case and the the other extreme case –scenario (B), when all the remaining fitness are gone when the final defence is breached. The results are the same for these two extreme cases, so can be extended to the other intermediate cases when the prey still can reproduce (positive remaining fitness) but the remaining fitness is diminished when the final defence is breached.

If plant defences do offer sequential barriers to herbivores, what can our model tell us about variation in investment in these defences? Some insight is possible here from the notable meta-analysis of studies in herbivore damage reported by Carmona *et al.* [5]. They report that variation in concentrations of plant secondary metabolites is a poor predictor of herbivore damage overall. In contrast, variation in physical defences, such as hairs and spines, provided better overall prediction of damage. The most consistent predictor of herbivore damage was however in life history traits, such as varied phenology which allow growth and flowering at times that enemies are rare - effectively hiding in time. One interpretation of these results is that it supports the sequential nature of plant defences, with the earlier acting defences (hiding, then physical defences) having much stronger influence on vulnerability than the last line of defence, of plant tissues by toxic secondary metabolites. If this interpretation has general validity, then it suggests that our framework can have widespread application in plant-animal interactions. Several items need to be measured for parameterised evaluation of the model's predictions including; costs of generating and deploying defences, survival benefits of each defence. In principle however, the model is open to empirical testing, and in the right systems may even be open to testing through experimental evolution. Key predictions could then be tested, for example that chemical defences never have more investment than earlier acting physical defences. We note the complexity of ontogenetic choice by plants makes the area all the

more interesting (see ref. [8]), and suggests developments of our approach to incorporate developmental plasticity.

2.4.2 Relation to Other Theoretical Work on Sequential Defences

We present here a general model to predict the optimal investment in sequential defences. We now consider our model's relevance to other, often more specialised models of defence. Our work here can be seen as a generalisation of the work of Broom *et al.* [9], who presented a simple model of investment across two sequentially encountered anti-predatory defences. A predator must overcome both defences to capture the prey, and probability of overcoming a defence declines linearly with increasing investment in defences. However there are costs every time a defence is used and these increase linearly with investment in a particular defence. On top of that there is an initial outlay in the construction of a defence, with the fecundity of the prey being a decreasing decelerating function of investment across both defences.

Broom *et al.* [9] provide predictions for circumstances where there is investment in only one defence or investment spread across both defences. When the ratio of the constitutive costs to the effectiveness of defences is generally similar and low for both defences, then investment across both defences can be optimal. Increasing rate of attack also increases the likelihood of investment across both defences. However investment in both defences was only predicted for relatively narrow combinations of circumstances, where investing heavily to produce one very effective defence was prohibitively expensive and the best solution was to offer two modestly effective defences that must be overcome. Our model further solves the problem where there are more than two defences, and gives the conditions under which investment are applied in multiple defences or only one defences, and the relation between investment in the sequential defences.

Strategy Blocking

The host reed warbler *Acrocephalus scirpaceus* is often found to have an egg-rejection defence strategy but not a chick-rejection defence strategy against the parasite cuckoo *Cuculus canorus*. Britton *et al.* [10] uses a concept called "strategy blocking" to explain this phenomenon. Strategy blocking describes the situation in which a strategy which would be adaptive in isolation ceases to be adaptive in the presence of a second strategy.

Strategy blocking explains this phenomenon in terms of the different pay-offs for each defence, but it is not framed as a sequential defences scenario, so it does not explain the effect of the sequence on defence strategy. Our sequential defences model provides an alternative explanation for why the reed warblers are found more likely to have defences in the earlier stage (rejecting the eggs) than in the later stage (rejecting the chicks). We particularly consider the condition under which the investment will be concentrated only in the first defence (egg-rejection). The rate that the warblers fail or succeed in rejecting the cuckoos' eggs (which corresponds to s_1 in our model) is dependant on the investment in the egg-rejection defence, which could explain why warblers are sometimes found not to reject eggs.

Although the concept of strategy blocking is raised in a population dynamics model [10], its idea that one defence will often reduce the benefits of a second defence can be explained otherwise through probabilities. Let us assume that if a predator encounters a prey then it is repelled with probability a if defence A only is expressed by the prey, with probability b if defence B only is expressed and with probability $1-(1-a)(1-b)$ if both are expressed. This implies that the two defences work independently and the predator must overcome all defences expressed in order to be successful. The benefit of defence B is the increase in the probability of an predator being repelled when defence B is expressed relative to when B is not expressed. This is a function of a, the higher the value of a (the more predators would have been repelled without B being expressed by defence A), the less often investing in B makes a difference to the prey and so the less the benefit of investment in B. This was a situation where the two defences worked independently, but it may also be the case that expression of one defence reduces the effectiveness of another, in our case that increasing a causes a decrease in b. As an example, if an animal invests in a heavy armoured shell, then its ability to outrun predators is compromised. The work of Britton *et al.* [10]

can be seen as a more general examination of earlier modelling by Brodie *et al.* [11] that reached essentially similar conclusions in a more restricted setting.

In contrast Kilner and Langmore [12] introduce the concept of *strategy-facilitation* as the complement to the concept of *strategy-blocking*. Here they imagine that the evolution of one defence makes the evolution of another defence easier. As an example of this they cite the modelling work of Svennungsen and Holen [13] who demonstrated that in avian brood parasite systems it can sometimes be advantageous for hosts to reject a randomly-selected

egg if they know that they have been parasitised but are unable to identify the parasitic egg. Kilner and Langmore [12] argue that if the strategy of such random rejection evolves then this will allow subsequent evolution of egg recognition to facilitate non-random targeting of the alien egg. As well as facilitating cognitive changes in the host it could trigger physiological change in egg appearance to improve such recognition.

This means that sometimes an inefficient defence is worth employing/investing in; in our model example (equation (2.32)) this is a defence with a high value of k_i. Given this defence is invested in, its cost has an effect on the fitness function R, which if it was not invested in ($s_i = 0$) would be absent. There is thus evolutionary pressure to improve the efficiency (lower k_i) if this were possible, which there would not be in the absence of investment.

Kilner and Langmore [12] also argue that defences can operate at levels of organisation greater than the individual that are often overlooked. They give as an example workers of the ant genus *Temnothorax* that can be enslaved by the species *Protomognathus americanus*, but which selectively destroy the slave-making pupae in their care. As a result *P. americanus* colonies are unusually small for a slavemaker and are less effective at conducting slave raids on neighbouring *Temnothorax* colonies. Since *Temnothorax* populations are highly kin-structured then there is a kin-selected benefit to this defence. Kilner and Langmore [12] speculate that as a generality kin-structuring in a host population will select for a more extensive portfolio of defences. They also predict that a high parasitic virulence will also select for more extensive portfolios. The last of their predictions is that where a parasite exploits more than one host, competition between the hosts to shift their parasite's attention toward the others should again select for complexity of defensive portfolio.

Coevolutionary Considerations in Sequential Defence Suites

Jongepier *et al.* [14] argue that for sequential lines of defence, later lines will be more expensive. Thus arms races between prey and predators would have started with the prey using a cheap defence acting early in the predation sequence, but as the predator evolved to overcome this defence there would then have been selection pressure for investment in later-acting more costly defences. Thus over evolutionary time there will have been a shift towards investment in more costly defence that act later in the sequence of the interaction between prey and predator. To put this a different way, the temporal order in which defences are employed will reflect the order in which they evolved. Gilman *et al.* [15] ar-

gue theoretically that there are co-evolutionary advantages to a multi-dimensional defence against any type of antagonist (parasite, predator or pathogen). Using a modelling framework, they argue that a prey is more likely to evolve a way to neutralise the predator as the number of defences increases or as the correlation between values across traits increases. Essentially each additional trait provides the prey with an additional opportunity to evolve an effective escape mechanism. A key point here is that sequential layering of defences is not necessary for these general conclusions, rather it is the use of multiple defences per se that matters. This is illustrated in Gilman *et al.*'s [15] model itself, and in a subsequent extension modelling plant toxicity by Speed and Ruxton [16]. Sasaki [17] considers the multiplicative interaction among the effect of defence genes, and finds that the cost of resistance and virulence values can influence the coexistence of multiple defences in static equilibria or coevolutionary cycle.

In contrast, Beatman *et al.* [18] introduce population dynamics into the discussion of investment across defences. They use a two-prey, one-predator Rosenzweig-McArthur model of predator-prey interaction. Prey can invest in each of two defences, one of which acts before the other in the predation sequence, and defences have costs as well as anti-predatory benefits. The system is allowed to come to equilibrium with only a single prey before a different prey with a different investment strategy across defences is introduced at low population density. Beatman *et al.* [18] then explore whether this second prey increases in population size. They conclude that the invasion of a given defence strategy is dependent on the fine detail of traits of the predator and the existing prey type, and the nature of the costs and benefits of the different defences; and so general conclusions are difficult to draw. However they do conclude that on the basis of their simulations "there exists no exclusive ecological or evolutionary advantage to defending early in the predation sequence". The word "exclusive" seems important here they mean there is nothing fundamentally beneficial about easy disruption of attacks per se from a population dynamic perspective. We agree with this, but there are mechanisms (like risk of injury or time lost to other beneficial activities) that may be correlated with early disruption, are not considered in their model, and bring benefits.

2.5 Conclusions

In our view the sequential organisation of defences has received relatively little rigorous examination in the literature. This is explained in part by expertise focusing on the mechanisms of individual defensive types (e.g. camouflage or chemical defence), rather than their integration into suites of defences. A valuable predictive aspect of our model, is to make a general argument that explains why earlier defences may gain higher investment than later acting defences. Suppose that a victim could biologically generate a suite of ten equally effective sequential defences, but it is optimal to only invest in five, then which five should it invest in, and how much in each? Our model predicts that the solution is to concentrate in earliest five defences rather than in the other five defences. Moreover, regarding the trade-off of the investments among each defence, a victim will invest no less in earlier defences than later defences, given that the investment functions among defences are the same (e.g. converting resources into defences is equally efficient across the sequential defences). In our Discussion section, we have shown that the model can be applied to animal, plant and other defensive systems. Our model can replicate and add quantitative rigor to the question of strategy-blocking, in which the effectiveness of early-acting defences makes the deployment of later acting defences redundant. In relating it to other theoretical works in the field, we note that coevolutionary approaches to the general question we examine here would add predictive sophistication.

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2.6 Appendices

2.6.1 Appendix A

The relation between s_i and s_{i+1} when $c_{i+1} = 0$

(i) We will prove that when $c_{i+1} = 0$, and when $(1-s)I'(s)$ is an increasing function, only $s_i = s_{i+1}$ can happen.

(a) When both $s_i > 0$ and $s_{i+1} > 0$, and from (2.25) and $c_{i+1} = 0$ we would have $(1 - s_{i+1})I'(s_{i+1}) = (1 - s_i)I'(s_i)$ and so $s_i = s_{i+1}$.

(b) and when both $s_i = 0$ and $s_{i+1} = 0$, we have $s_i = s_{i+1}$.

(c) If $s_i > s_{i+1} = 0$, from the necessary condition (2.15) and (2.16), we have

$$\begin{aligned}
&\frac{\partial R}{\partial s_i} \cdot (1 - s_i) - h(1 - s_i)I'(s_i) \\
&= \prod_{j=1}^{n}(1 - s_j)\big(1 - C(I_A) - \sum_{j=1}^{n} c_j\big) - (1 - s_i)(1 - \prod_{j=1}^{n}(1 - s_j))\frac{\partial C(I_A)}{\partial s_i} + \sum_{j=i+1}^{n} c_j \prod_{k=1}^{j-1}(1 - s_k) \\
&- h(1 - s_i)I'(s_i) \\
&= 0, \qquad \text{(A.1)}
\end{aligned}$$

$$
\begin{aligned}
&\frac{\partial R}{\partial s_{i+1}} \cdot (1-s_{i+1}) - h(1-s_{i+1})I'(s_{i+1}) \\
&= \prod_{j=1}^{n}(1-s_j)\big(1-C(I_A)-\sum_{j=1}^{n}c_j\big) - (1-s_{i+1})(1-\prod_{j=1}^{n}(1-s_j))\frac{\partial C(I_A)}{\partial s_{i+1}} + \sum_{j=i+2}^{n} c_j \prod_{k=1}^{j-1}(1-s_k) \\
&- h(1-s_{i+1})I'(s_{i+1}) \\
&\leq 0 \qquad\qquad (A.2)
\end{aligned}
$$

Then following are the similar deduction as (2.22)-(2.27), we have

$$
\begin{aligned}
&- (1-s_{i+1})(1-\prod_{j=1}^{n}(1-s_j))\frac{\partial C(I_A)}{\partial s_{i+1}} + \sum_{j=i+2}^{n} c_j \prod_{k=1}^{j-1}(1-s_k) - h(1-s_{i+1})I'(s_{i+1}) \\
&\leq -(1-s_i)(1-\prod_{j=1}^{n}(1-s_j))\frac{\partial C(I_A)}{\partial s_i} + \sum_{j=i+1}^{n} c_j \prod_{k=1}^{j-1}(1-s_k) - h(1-s_i)I'(s_i). \qquad (A.3)
\end{aligned}
$$

Since $I_A = \sum_{i=1}^{n} I(s_i)$, the above is equivalent to

$$
\begin{aligned}
&- (1-s_{i+1})(1-\prod_{j=1}^{n}(1-s_j))C'(I_A)I'(s_{i+1}) - h(1-s_{i+1})I'(s_{i+1}) \\
&\leq -(1-s_i)(1-\prod_{j=1}^{n}(1-s_j))C'(I_A)I'(s_i) + c_{i+1}\prod_{k=1}^{i}(1-s_k) - h(1-s_i)I'(s_i). \qquad (A.4)
\end{aligned}
$$

$\Rightarrow$

$$
\begin{aligned}
&- (1-s_{i+1})I'(s_{i+1})\Big((1-\prod_{j=1}^{n}(1-s_j))C'(I_A) + h\Big) \\
&\leq -(1-s_i)I'(s_i)\Big((1-\prod_{j=1}^{n}(1-s_j))C'(I_A) + h\Big) + c_{i+1}\prod_{k=1}^{i}(1-s_k). \qquad (A.5)
\end{aligned}
$$

Since $C'(I_A) > 0$ and also $1-\prod_{j=1}^{n}(1-s_j) > 0$ and $h \geq 0$, we have that $\Big((1-\prod_{j=1}^{n}(1-$

$s_j))C'(I_A)+h\Big) > 0$, so that equation (2.24) is equivalent to

$$-(1-s_{i+1})I'(s_{i+1}) \leq -(1-s_i)I'(s_i) + c_{i+1}\frac{\prod_{k=1}^{i} s_k}{\Big((1-\prod_{j=1}^{n}(1-s_j))C'(I_A)+h\Big)}. \quad (A.6)$$

The last term in the right-hand side $c_{i+1}\frac{\prod_{k=1}^{i} s_k}{\Big((1-\prod_{j=1}^{n}(1-s_j))C'(I_A)+h\Big)}$ is positive when $c_{i+1} > 0$, therefore

$$-(1-s_{i+1})I'(s_{i+1}) \leq -(1-s_i)I'(s_i). \quad (A.7)$$

which is the same to,

$$(1-s_{i+1})I'(s_{i+1}) \geq (1-s_i)I'(s_i). \quad (A.8)$$

(35) together with that the function $(1-s)I'(s)$ is increasing violate that $s_i > s_{i+1} = 0$. So $s_i > s_{i+1} = 0$ is not possible.

(d) $s_{i+1} > s_i = 0$ is not possible either for the same reason as (c).

Therefore, when $c_{i+1} = 0$ and when $(1-s)I'(s)$ is an increasing function, we have $s_i = s_{i+1}$ (Example see in Figure 2.2).

(ii) We will prove that when $c_2 = 0$, and when $(1-s)I'(s)$ is a decreasing function, only $s_1 > s_2 = 0$ or $s_2 > s_1 = 0$ can happen.

(a) When both $s_1 > 0$ and $s_2 > 0$, and when $c_2 = 0$, from (2.25),

$$(1-s_{i+1})I'(s_{i+1}) = (1-s_i)I'(s_i). \quad (A.9)$$

so we have $s_i = s_{i+1}$.

However, when $s_1 = s_2 = 1-m$, for some specific $m \in (0,1)$, we can always increase the value of R by decreasing the value of s_1 and increasing the value of s_2, given that $(1-s_1)(1-s_2) = m^2$ and the values of the other s_j $(j > 2)$ fixed (e.g. let $s_1 = 1-m^{\frac{1}{2}}$

and $s_2 = 1 - m^{\frac{3}{2}}$). This is because, the solution that $s_1 = s_2 = 1 - m < 1$ is the maximum solution of $I(s_1) + I(s_2)$ given that $(1 - s_1)(1 - s_2) = m^2$ and therefore the minimum solution of R (see in (2.4)) given that $(1 - s_1)(1 - s_2) = m^2$ and the values of the other s_j $(j > 2)$ fixed.

To prove this, we only need to see the necessary and sufficient condition for the question

$$Max \quad I(s_1) + I(s_2) \quad s.t.(1 - s_1)(1 - s_2) = m^2 \tag{A.10}$$

that is

$$Max \quad F(s_1) = I(s_1) + I(1 - \frac{m^2}{1 - s_1}), \tag{A.11}$$

where m^2 is a constant value.

The necessary condition (the first derivative of $F(s_1)$ equals 0) is that

$$\begin{aligned} F'(s_1) &= I'(s_1) + I'(1 - \frac{m^2}{1 - s_1})(-\frac{m^2}{(1 - s_1)^2}) \\ &= I'(s_1) + I'(s_2)(-\frac{1 - s_2}{1 - s_1}) \\ &= 0 \end{aligned} \tag{A.12}$$

which is equilalent to

$$(1 - s_1)I(s_1) = (1 - s_2)I(s_2), \tag{A.13}$$

so when $(1 - s)I'(s)$ is decreasing,

$$s_1 = s_2 \tag{A.14}$$

The sufficient condition (the second derivative of $F(s_1)$ larger than 0) is that

$$\begin{aligned} F''(s_1) &= I''(s_1) + I''(1 - \frac{m^2}{1-s_1})\frac{m^4}{(1-s_1)^4} - 2I'(1 - \frac{m^2}{1-s_1})\frac{m^2}{(1-s_1)^3} \\ &= \frac{1}{(1-s_1)^2}\Big((1-s_1)^2 I''(s_1) + (1-s_2)^2 I''(s_2) - 2(1-s_2)I'(s_2)\Big) \\ &= \frac{1}{(1-s_1)^2}\Big(2(1-s_2)^2 I''(s_2) - 2(1-s_2)I'(s_2)\Big) \quad (\text{since } s_1 = s_2) \\ &< 0 \quad (\text{since } (1-s)I'(s) \text{ is decreasing function}) \end{aligned} \tag{A.15}$$

So $s_1 = s_2 > 0$ is the local maximum solution of $I(s_1) + I(s_2)$ and therefore the local minimum solution of R.

(b) When both $s_1 = 0$ and $s_2 = 0$, we can follow the proof below in (iii)(b)(c), and then all rest $s_i = 0$ $(i > 2)$, which is not the optimal solution for R.

Therefore, when $c_2 = 0$, and when $(1-s)I'(s)$ is a decreasing function, only $s_1 > s_2 = 0$ or $s_2 > s_1 = 0$ can be the optimal (Example see in Figure 2.4).

(iii) We will prove that when $(1-s)I'(s)$ is a decreasing function, for any $i \neq 2$, $c_i = 0$ but $c_2 > 0$ does not change the relation $1 > s_1 > s_2 = ... = s_n = 0$ (equation (2.28)).

(a) $c_1 = 0$ does not change the relation in (2.25), therefore the relation (2.28) still holds.

(b) If $c_3 = 0$ but $c_2 > 0$, we would have $s_1 > 0$ and $s_2 = 0$ since $c_2 > 0$. If however $s_3 = m > 0$, since the symmetric relation between s_2 and s_3 in R when $c_3 = 0$, we would have $s_2 = m > 0$ and $s_3 = 0$ to be another optimal solution, which violates the fact that $s_2 = 0$. So s_3 can only be 0.

If $c_3 > 0$, s_3 can still only be 0 due to the asymmetric relation between s_2 and s_3 in the R function and that we can only have $s_2 \geq s_3$.

Therefore, no matter $c_3 > 0$ or $c_3 = 0$, we can only have $s_3 = 0$

(c) For the same reason, no matter $c_4 > 0$ or $c_4 = 0$, we can only have $s_4 = 0$; and so is for any $c_i = 0$ $(i > 2)$.

Therefore when for any $i \neq 2$, $c_i = 0$ but $c_2 > 0$ and when $(1-s)I'(s)$ is a decreasing function, the relation (2.28) still holds.

2.6.2 Appendix B

When the residual fitness when all the defences are breached is positive (scenario (A)), the fitness function is (2.3) as follows.

$$R(s_1, s_2, \ldots, s_n) = 1 - C(\sum_{i=1}^{n} I_i(s_i)) - c_1 - \sum_{j=2}^{n} c_j \prod_{k=1}^{j-1}(1-s_k). \tag{B.1}$$

The analysis for (2.3) is similar to when the fitness function is (2.4) (scenario (B)– when the residual fitness is zero when all the defences are breached). The necessary conditions to maximise R– (2.15) and (2.16) in this case can be written as follows,

(I) When $0 < s_i < 1$,

$$\frac{\partial R}{\partial s_i} - hI'(s_i) = -\frac{\partial C(I_A)}{\partial s_i} + \frac{1}{1-s_i}\sum_{j=i+1}^{n} c_j \prod_{k=1}^{j-1}(1-s_k) - hI'(s_i) = 0$$
$$(\text{with } h \geq 0) \tag{B.2}$$

(II) When $s_i = 1$,

$$\frac{\partial R}{\partial s_i} - hI'(s_i) = -\frac{\partial C(I_A)}{\partial s_i} - \frac{1}{1-s_i}\sum_{j=i+1}^{n} c_j \prod_{k=1}^{j-1}(1-s_k) - hI'(s_i) \leq 0$$
$$(\text{with } h \geq 0) \tag{B.3}$$

Now (B.2) and (B.3) together is the necessary condition. The following analyses are similar to the scenario (B). Similar to the equations (2.20) and (2.21),

$$\frac{\partial R}{\partial s_i} \cdot (1 - s_i) - h(1 - s_i)I'(s_i) = -(1 - s_i)\frac{\partial C(I_A)}{\partial s_i} + \sum_{j=i+1}^{n} c_j \prod_{k=1}^{j-1}(1 - s_k) - h(1 - s_i)I'(s_i) = 0, \tag{B.4}$$

$$\frac{\partial R}{\partial s_{i+1}} \cdot (1 - s_{i+1}) - h(1 - s_{i+1})I'(s_{i+1}) = -(1 - s_{i+1})\frac{\partial C(I_A)}{\partial s_{i+1}} + \sum_{j=i+2}^{n} c_j \prod_{k=1}^{j-1}(1 - s_k) - h(1 - s_{i+1})I'(s_{i+1}) = 0 \tag{B.5}$$

Then we have

$$-(1 - s_{i+1})\frac{\partial C(I_A)}{\partial s_{i+1}} + \sum_{j=i+2}^{n} c_j \prod_{k=1}^{j-1}(1 - s_k) - h(1 - s_{i+1})I'(s_{i+1}) = -(1 - s_i)\frac{\partial C(I_A)}{\partial s_i} + \sum_{j=i+1}^{n} c_j \prod_{k=1}^{j-1}(1 - s_k) - h(1 - s_i)I'(s_i). \tag{B.6}$$

Since $I_A = \sum_{i=1}^{n} I(s_i)$, the above is equivalent to

$$-(1 - s_{i+1})C'(I_A)I'(s_{i+1}) - h(1 - s_{i+1})I'(s_{i+1}) = -(1 - s_i)C'(I_A)I'(s_i) + c_{i+1}\prod_{k=1}^{i}(1 - s_k) - h(1 - s_i)I'(s_i). \tag{B.7}$$

$\Rightarrow$

$$-(1 - s_{i+1})I'(s_{i+1})\Big(C'(I_A) + h\Big) = -(1 - s_i)I'(s_i)\Big(C'(I_A) + h\Big) + c_{i+1}\prod_{k=1}^{i}(1 - s_k). \tag{B.8}$$

Since $C'(I_A) > 0$, we have that $\Big(C'(I_A) + h\Big) > 0$, so that equation (B.8) is equivalent to

$$-(1 - s_{i+1})I'(s_{i+1}) = -(1 - s_i)I'(s_i) + c_{i+1}\frac{\prod_{k=1}^{i}(1 - s_k)}{\Big(C'(I_A) + h\Big)}. \tag{B.9}$$

The last term in the right-hand side $c_{i+1}\frac{\prod_{k=1}^{i}(1-s_k)}{\Big(C'(I_A)+h\Big)}$ is negative since $c_{i+1} > 0$, $C'(I_A) > 0$ and $h \geq 0$, therefore

$$-(1 - s_{i+1})I'(s_{i+1}) > -(1 - s_i)I'(s_i). \tag{B.10}$$

which is equivalent to,

$$(1 - s_{i+1})I'(s_{i+1}) < (1 - s_i)I'(s_i). \tag{B.11}$$

Therefore we have the same relation between s_i and s_{i+1} as (2.27). The later analyses are the same as in the section "Investing in multiple defences or in a single defence?".

Chapter 3

The evolution of variance in sequential defences

3.1 Introduction

Protective defences against organisms' enemies, such as predators, parasites and pathogens, are ubiquitous [1, 2, 3, 4, 5, 6] and the study of adaptations for defence is consequently a major theme in adaptive evolutionary biology.

Evolutionary studies of defences often focus on one or more perspectives, including: the evolutionary history of defence mechanisms [3, 7, 8], their roles in macroevolutionary patterns [9, 10, 11], the variety of forms of defences used in taxonomic groups [1, 12], the influence of life-history variation on defence [13, 14, 15, 16, 17], coevolution [18, 19] and strategies for optimal investment in defences [20, 21].

Despite the extensive research in the biology of defence, an area that has received relatively little attention is the nature of defensive variation between individuals and between species. Thus, many studies which seek to understand the function and mode of action of defensive phenotypes focus (rightly) on species typical defences, rather than variation within species. The notable exception to this is seen when frequency dependent evolution causes stable polymorphisms in defences, for example those that give the greatest net benefit when rare, such as parasitic Batesian mimicry [4]. Some classes of defence are however, very variable

within populations. Chemical defences of plants [22] and animals [23] are, for example, notoriously variable, both in terms of the concentrations of compounds that can repel and deter predatory enemies, and even in the mixtures of compounds that are present in different individuals [23]. Arguably, less is known about variation in other forms of defence in animals such as camouflage or warning signals, because of an emphasis on species-typical traits. However, the recent onset of methods for measuring colour patterns is enabling some evaluation of levels of variation in animal colouration, but overall conclusions cannot be made at present. Similarly, chemical ecologists have for a long period been able to evaluate (and demonstrate) variation in secondary metabolites in plants [24]. In addition variation in physical defences (density of protective trichome hairs, thickness of cuticles and waxes etc), can be measured, and reveal the level of variation there is within populations [25].

One reason for the interest in the variation of defences is, as described above, that they can be very variable indeed. There is an apparent paradox here; traits that are viewed as vital to survival of individuals are none the less highly variable, suggesting that some individuals are poorly protected in populations. Several explanations have been proposed including frequency dependence (rare toxins work best) because of predator-counter adaptation and coevolution [26]. A second compelling explanation is that the effectiveness of some forms of defence saturate at levels that are phenotypically cheap to achieve by organisms, hence a lot of observed variation is above a threshold of effectiveness-saturation, of little effect on survival and with little variation in costs between individuals [23]. It might be for example that some defensive chemicals are cheaply synthesised and stored, and the observed levels of variation in concentration imply nothing about variation in survival from attack.

Here we propose an additional and potentially predictive explanation for different levels of variation in different kinds of defence.

We reason that many defences often work in what Frank [27] calls “sequential layers”. Defences are in effect ordered as a set of barriers surrounding the organism: each one must be crossed in turn by an enemy before it can reach the valuable core tissues of the victim. As Endler [28] and Broom *et al.* [20] point out, those components of sequential defence suites which are met first will be challenged by enemies more frequently than those that are met only later in a sequence. A general conclusion is then that those defences met or deployed early in an encounter with an enemy will have a larger contribution to the protection of a victim than those met later. Suppose we have two defences that act in

sequence, and the probability that an enemy successfully crosses each is 0.5. For each time the first defence is challenged, the second defence is challenged only half as often, and its contribution to survival is half that of the first.

Put simply then, selection is likely to be weaker on later acting, than on earlier acting defences. We may then predict that the mutation-selection equilibrium for a defensive trait is different depending at what stage in encounters with an enemy it is deployed. For example, an organism whose only defence is chemical in nature relies very strongly on that defence and selection to keep it at an optimal value will be very strong. Should however the organism evolve an effective physical defence that acts before the chemical defence, then the chemical defence is used less often and makes a smaller contribution to survival from an encounter. The "corrupting" effects of mutation will make more headway against the unifying force of selection toward the optimal value of the trait.

Though it is easy to argue this verbally, here we seek a quantitative analysis to evaluate the effects of order of deployment on mutation. We present a model that is simple in structure (with only two stages) and investigates the dynamical evolution of paired, sequential defences, seeking out the conditions in which there will be inequalities in variation between them arising from mutation-selection balance. A key point is that while we do confirm that the later acting defence may often evolve to be more variable, we can identify conditions in which the later-acting defences are the least variable.

Several other theoretical papers look at sequential defences, and though none focus on the question we ask here about variability, we will briefly comment on their relevance to our model here.

Broom *et al*'s sequential defence model [20] gave different benefit and cost values to both defences, and found the optimal strategies (none/preattack defence/post attack defence/both defences) in regards to these different benefit and cost values. In the model due to the order in defence, the relation of benefits and costs of the first defence can influence the condition when the second defence is used or not; but the relation of benefits and costs of the second defence can not influence the condition when the first defence is used or not. So the first defence might be relatively more influential in the optimal decision making.

Speed *et al* [23], Gilman *et al* [19] and Sasaki [29] gave coevolutionary models to explore the investments in different defences. In Speed *et al* [23], victims could invest in one or

more defences, and coevolution could be the reason for more than one defence, since when there is not coevolution, plants evolve to invest in only one toxin trait. Gilman *et al* [19] 's paper showed that increasing the number of defence traits, and the correlation between traits could help the victims to win the evolutionary contest, so different defence traits functioning interactively might be the reason why more than one defence is profitable. Sasaki [29] found that when the effects of defence genes acts multiplicatively, different resistant defences exist in either coevolutionary cycle or static equilibria depending on the cost of resistance and virulence values. These models gave reasons for the existence of more than one defences, although these models did not show the defence variance evolution of each defences as we did.

3.2 The Model

We consider a prey species that mounts two sequential defences against predation. We assume that each individual has a phenotype x describing its first-level defense and a phenotype y characterising its second-level defense. These phenotypes determine the success of each defense repelling predation, so not breached by enemies, and we denote by $p_1(x)$ the probability that the first defense holds and by $p_2(y)$ the probability that the second defense holds. We assume that there are ideal values a and b for these phenotypes, so that $p_1(x)$ is maximal at $x = a$, and $p_2(y)$ is maximal when $y = b$, and that the defense will be less likely to hold when the phenotypic values are further away from these ideal values. Specifically, we assume functional forms

$$p_1(x) = e^{-\epsilon_1 - \frac{(x-a)^2}{\alpha}}, \tag{B.1}$$

$$p_2(y) = e^{-\epsilon_2 - \frac{(y-b)^2}{\beta}}, \tag{B.2}$$

so that the first (respectively, second) defense will hold with probability $e^{-\epsilon_1}$ (respectively, $e^{-\epsilon_2}$) when the corresponding phenotype is at its ideal value $x = a$ (respectively, $y = b$), and that the tolerance of phenotypic deviations from the ideal will be wide when α (respectively, β) is large.

Since these defenses are met sequentially, there are three mutually exclusive scenarios: (1) defense 1 holds, which occurs with probability $p_1(x)$; (2) defense 1 fails, but defense 2 holds, which occurs with probability $(1 - p_1(x))p_2(y)$; (3) defenses 1 and 2 both fail,

which occurs with probability $(1-p_1(x))(1-p_2(y))$. We assume that the prey's fitnesses under these three scenarios are f_1, f_2 and f_3 respectively, and note that these represent increasingly adverse outcomes for the prey so that $f_1 \geq f_2 \geq f_3 \geq 0$. The average fitness of an individual with phenotype (x, y) is then given by

$$\Phi(x,y) = f_1 p_1(x) + f_2(1-p_1(x))p_2(y) + f_3(1-p_1(x))(1-p_2(y)) \tag{B.3}$$

We now consider how the population distribution of the phenotypes evolves in time. We assume non-overlapping generations, and let $N_t(x_t, y_t)$ represent the density of individuals with vector of phenotypes (x_t, y_t) at generation t. We first consider the case where the phenotype is completely heritable with no mutation. In that case, the abundance of individuals with phenotypes (x_t, y_t) simply changes by $\Phi(x_t, y_t)$ at each generation:

$$N_{t+1}(x_{t+1}, y_{t+1}) = N_t(x_t, y_t)\Phi(x_t, y_t). \tag{B.4}$$

Secondly, we consider the case where phenotype mutates between generations. If $M(x_t, y_t, x_{t+1}, y_{t+1})$ is the mutation kernel, i.e. the probability density that a parent with phenotype (x_t, y_t) has offspring of phenotype (x_{t+1}, y_{t+1}), then

$$N_{t+1}(x_{t+1}, y_{t+1}) = \int_{-\infty}^{+\infty}\int_{-\infty}^{+\infty} N_t(x_t, y_t)\Phi(x_t, y_t)M(x_t, y_t, x_{t+1}, y_{t+1})dx_t\, dy_t. \tag{B.5}$$

We assume that the phenotypes mutate independently with a Gaussian mutation kernel of the form

$$M(x_t, y_t; x_{t+1}, y_{t+1}) = \frac{1}{\pi\mu} e^{-\frac{(x_t - x_{t+1})^2 - (y_t - y_{t+1})^2}{\mu}}, \tag{B.6}$$

so that $\mu/2$ is the variance in the mutation per generation in either phenotype.

We assume that, at the first generation, the phenotypes are independently normally distributed with variances $v_1/2$ and $w_1/2$ and means $\bar{x}_1$ and $\bar{y}_1$ for the first and second defenses

respectively, and n_1 the total population number.

$$N_1(x_1, y_1) = n_1 \frac{1}{\pi\sqrt{v_1 w_1}} e^{-(\frac{(x_1-\bar{x}_1)^2}{v_1} + \frac{(y_1-\bar{y}_1)^2}{w_1})}. \tag{B.7}$$

The evolution of the distribution of phenotypes in the population is therefore obtained by starting with the initial distribution given in eqn. (B.7) and iterating eqns (B.4) or (B.5), substituting for Φ from eqn. (B.3), p_1 and p_2 from eqns. (B.1) and (B.2), and M from eqn. (B.6). We characterise the population distribution by the means $\overline{x}_t$ and $\overline{y}_t$ and variances $\frac{v_t}{2}$ and $\frac{w_t}{2}$ of the phenotypic values, defined as

$$\begin{aligned}
\overline{x}_t &= E_t(x_t) \\
\overline{y}_t &= E_t(y_t) \\
\frac{v_t}{2} &= E_t((x - \overline{x}_t)^2) \\
\frac{w_t}{2} &= E_t((y - \overline{y}_t)^2),
\end{aligned}$$

where $E_t(\cdot)$ represents the expectation value at time t, defined as

$$E_t(f(x_t, y_t)) = \frac{\int_{-\infty}^{\infty}\int_{-\infty}^{\infty} f(x_t, y_t) N_t(x_t, y_t) dx_t dy_t}{\int_{-\infty}^{\infty}\int_{-\infty}^{\infty} N_t(x_t, y_t) dx_t dy_t}$$

for any function $f(x, y)$. Note that the total number of population at t,

$$n_t = \int_{-\infty}^{\infty}\int_{-\infty}^{\infty} N_t(x_t, y_t) dx_t dy_t.$$

Also note that the scale of the variance in defence phenotypes is set by the parameters μ, α, and β. That is, if we increase these three parameters by a common factor, the equilibrium value of v_t and w_t will change by the same factor (Appendix B).

To summarise, the parameters, variables and functions are shown in the tables 4.2,4.1,3.3.

Parameters	
μ	mutation strength
a, b	ideal phenotypes
ϵ_1, ϵ_2	effectiveness
α, β	Tolerance of phenotypic deviations from the ideal
f_1, f_2, f_3	conditional fitness

Table 3.1: Parameter table

Variables	
x_t, y_t	defence phenotype values at time t
$\bar{x}_t, \bar{y}_t$	means of defence phenotype values at time t
$\frac{v_t}{2}, \frac{w_t}{2}$	variances of defence phenotype values at time t
n_t	total population numbers at time t

Table 3.2: Variable table

Functions	
$p_1(x), p_2(y)$	probability each defence holds
$\Phi(x, y)$	average fitness
$M(x_t, y_t; x_{t+1}, y_{t+1})$	Gaussian Mutation Kernal from (x_t, y_t) to (x_{t+1}, y_{t+1})
$N_t(x_t, y_t)$	population density function about (x_t, y_t)

Table 3.3: Function table

Note that the idea of dealing with selection and mutation in this model is similar to the quasispecies model [30], except that the model here deals with continuous transitions across time in comparison with the discrete transitions across time in quasispecies model.

3.3 Methods

We use both numerical and analytical approaches to explore how different factors affect the variances of both defences. The section 3.1 is the numerical approach and the section 3.2 is the analytical approach.

3.3.1 Numerical integration

We are not able to find exact closed-form analytical expressions for the mean or variance of the phenotypes at generation t, so we approximate the continuous distribution of phenotypic values by a discrete set and iterate (B.4) or (B.5) numerically. A each t we replace (x_t, y_t) by the grid of pairs of values $\{(x_{ti}, y_{tj}); i \in \{1, 2, \ldots, n\}, j \in \{1, 2, \ldots, n\}\}$, where

$$\begin{aligned} x_{ti} &= x_{t1} + (i-1)\Delta x, \qquad i \in \{1, 2, \ldots, n\} \\ y_{tj} &= y_{t1} + (j-1)\Delta y, \qquad j \in \{1, 2, \ldots, n\}. \end{aligned}$$

In all cases we start with a population with means $(\bar{x}_1, \bar{y}_1) = (1, 1)$ and variances $(v_1/2, w_1/2) = (2, 2)$, and total population number $n_1 = 10000$. The fitnesses are set to $(f_1, f_2, f_3) = (2, 1, 0.2)$, the ideal phenotypes to $(a, b) = (0, 0)$, the selection forces are $(\alpha, \beta) = (5, 5)$, and the grid is defined by $\Delta x = \Delta y = 0.2$, $n = 101$. All integrals are approximated as follows:

$$\int_{-\infty}^{+\infty} \int_{-\infty}^{+\infty} F(x_t, y_t) dx_t \, dy_t \approx \sum_{i=1}^{n} \sum_{j=1}^{n} F(x_{ti}, y_{tj}) \Delta x \Delta y,$$

for any function F. The grid of values extends over a range of $(n-1)\Delta x = (n-1)\Delta y = 20$ units, which (for the variance values under consideration) is sufficient for this finite sum to approximate the infinite range of integration. To avoid numerical overflow, after each iteration we replace N_t by

$$N_t(x_{ti}, y_{tj}) \to N'_t(x_{ti}, y_{tj}) = n_1 \frac{N_t(x_{ti}, y_{tj})}{\sum_{i=1}^{n} \sum_{j=1}^{n} N_t(x_{ti}, y_{tj}) \Delta x \Delta y},$$

so that the population is always normalised to contain $n_1 = 10000$ individuals; this does not affect our results, as we are only interested in the relative abundance of different phenotypes.

For the case where there is no mutation, we use $x_{i1} = y_{i1} = -9$ (so that x extends over the

range $\overline{x}_1 \pm 10$ and y over the range $\overline{y}_1 \pm 10$), and iterate eqn. (B.4) at this grid of values for 1000 generations.

For the case where there is mutation, we allow the range of integration to vary as the mean phenotypic values change to ensure that the range of phenotypic values in the population does not stray too close to (or beyond) the edges of the range of integration. To do this, at each generation we calculate $\overline{x}_t$ and $\overline{y}_t$, and then set

$$x_{t+1,1} = \overline{x}_t - \frac{(n-1)}{2}\Delta x$$
$$y_{t+1,1} = \overline{y}_t - \frac{(n-1)}{2}\Delta y,$$

so that the ranges of x and y grid values at the next generation are centred on $\overline{x}_t$ and $\overline{y}_t$ respectively. Since the grid of (x, y) values changes between generations, we need to determine the density of phenotypes evaluated on the current generation's grid from the density evaluated on the previous generation's grid. We do this by assuming that the density is constant within a range $(\pm\frac{\Delta x}{2}, \pm\frac{\Delta y}{2})$ from the points on the previous generation's grid. We set $\mu = 0.02$ and iterate eqn. (B.5) for 1000 generations.

3.3.2 Normal approximation

An alternative method for calculating approximately the evolution in time of the population is to use a moment closure assumption, which is a well established approximation method for stochastic systems that cannot be solved exactly. Moment closure assumes that the distribution of a random variable is well approximated by a particular parametric form (Whittle [31], and then derives (approximate) equations for the parameters of the distribution. Here, we perform a normal moment closure by assuming that the traits are normally distributed at a generation t, and then calculate the mean and covariance matrix at generation $t+1$ in terms of the mean and covariance at time t. While the trait will not in general be normally distributed (except at the first generation, where this is assumed), it is reasonable to assume that the iteration equations for the mean and covariance provide a good enough approximation to the true time evolution of the system for the purposes of understanding the general behaviour of the model. This is an uncontrolled approximation, by contrast with the direct numerical solution described in the previous section (which will describe the dynamics exactly in the limit where the integrals are approximated by

sums over a very large and very fine grid), but has the advantage of being much quicker to evaluate and therefore permits a much wider exploration of parameter space. We have tested our approximation scheme against simulation results, and find that it reproduces the patterns in the result well for a wide range of parameters.

We begin by assuming that the traits are normally distributed at time t, and write the distribution of traits as

$$N_t(x_t, y_t) = n_t \frac{\sqrt{|W_t|}}{\pi} \exp\Big(-(z_t - \bar{z}_t)^T W_t (z_t - \bar{z}_t)\Big), \tag{B.8}$$

where $z_t = \begin{pmatrix} x_t \\ y_t \end{pmatrix}$ is the vector of defence phenotypes, $\bar{z}_t = \begin{pmatrix} \bar{x_t} \\ \bar{y_t} \end{pmatrix}$ is the mean vector of defence phenotypes, and $W_t = (2\Sigma_t)^{-1}$, where

$$\Sigma_t = E_t \begin{pmatrix} x_t^2 & x_t y_t \\ x_t y_t & y_t^2 \end{pmatrix} - E_t \begin{pmatrix} x_t \\ y \end{pmatrix} E_t \begin{pmatrix} x_t & y_t \end{pmatrix}$$

is the covariance matrix for the trait.

We can find the population distribution at the next generation by applying the iteration equation (B.5), where Φ is defined by equations (B.1–B.3) and M from eqn. (B.6). After performing the integrals over (x_t, y_t) (the details are shown in Appendix A), this leads to

$$N_{t+1}(x_{t+1}, y_{t+1}) = n_t \sqrt{|W_t|} \Theta_t \sum_{j=1}^{4} \theta_{t,j} \frac{1}{2\pi\sqrt{|\Sigma_{t+1,j}|}} \exp\Big(-\frac{1}{2}(z_{t+1} - z'_{t+1,j})^T \Sigma_{t+1,j}^{-1} (z_{t+1} - z'_{t+1,j})\Big), \tag{B.9}$$

where

$$
\begin{aligned}
z'_{t+1,j} &= (W_t + F_j)^{-1}(W_t \bar{z}_t + F_j \hat{a}) \\
\Sigma_{t+1,j} &= \frac{1}{2}\Big(U^{-1} + (\Sigma_t^{-1} + F_j)^{-1}\Big) \\
\gamma_1 &= f_3 \\
\gamma_2 &= (f_1 - f_3)e^{-\epsilon_1} \\
\gamma_3 &= (f_2 - f_3)e^{-\epsilon_2} \\
\gamma_4 &= (f_3 - f_2)e^{-(\epsilon_1+\epsilon_2)} \\
F_1 &= \begin{pmatrix} 0 & 0 \\ 0 & 0 \end{pmatrix} \\
F_2 &= \begin{pmatrix} \frac{1}{\alpha} & 0 \\ 0 & 0 \end{pmatrix} \\
F_3 &= \begin{pmatrix} 0 & 0 \\ 0 & \frac{1}{\beta} \end{pmatrix} \\
F_4 &= F_2 + F_3 \\
U &= \begin{pmatrix} \frac{1}{\mu} & 0 \\ 0 & \frac{1}{\mu} \end{pmatrix} \\
\theta_{t,j} &= \frac{\gamma_j s_{t,j}}{\Theta_t \sqrt{|W_t + F_j|}} \\
\Theta_t &= \sum_{j=1}^{4} \frac{\gamma_j s_{t,j}}{\sqrt{|W_t + F_j|}} \\
s_{t,j} &= \exp\Big(\frac{1}{\mu}(W_t \bar{z}_t + F_j \hat{a})^T \Big((W_t + F_j)^{-1} + \mu I\Big) K_{t,j}^{-1}(W_t \bar{z}_t + F_j \hat{a}) - \bar{z}_t^T W_t \bar{z}_t - \hat{a}^T F_j \hat{a}\Big) \\
\hat{a} &= \begin{pmatrix} a \\ b \end{pmatrix}
\end{aligned}
$$

This shows that N_{t+1} is the sum of four normal distributions with different means and covariance matrices, so cannot be expressed as a single normal distribution. We can, however, use this expression to compute the mean and covariance of the traits at generation $t + 1$

(also see equations (A.9), (A.11) in Appendix A):

$$\begin{aligned}\bar{z}_{t+1} &= E_{t+1}(z_{t+1}) \\ &= \sum_{j=1}^{4} \theta_{t,j}(W_t + F_j)^{-1}(W_t\bar{z}_t + F_j\hat{a}) \qquad \text{(B.10)} \\ \Sigma_{t+1} &= E_{t+1}\begin{pmatrix} x_{t+1}^2 & x_{t+1}y_{t+1} \\ x_{t+1}y_{t+1} & y_{t+1}^2 \end{pmatrix} - E_t\begin{pmatrix} x_t \\ y \end{pmatrix} E_t\begin{pmatrix} x_t & y_t \end{pmatrix} \\ &= \frac{1}{2}U^{-1} + \sum_{j=1}^{4} \theta_{t,j}(\Sigma_t^{-1} + 2F_j)^{-1} + \sum_{j=1}^{4} \theta_{t,j} \cdot z'_{t+1,j} z'^{T}_{t+1,j} - \bar{z}_{t+1}\bar{z}^{T}_{t+1}. \qquad \text{(B.11)}\end{aligned}$$

From the expression of F_j ($j = 1, 2, 3, 4$), we can tell from eqn. (B.10) that the mean will evolve over time towards the ideal phenotype $\hat{a}$. Also $z'_{t+1,j} = (W_t + F_j)^{-1}(W_t\bar{z}_t + F_j\hat{a})$ ($j = 1, 2, 3, 4$) approaches to the ideal phenotype $\hat{a}$, therefore the term $\sum_{j=1}^{4} \theta_{t,j} \cdot z'_{t+1,j} z'^{T}_{t+1,j} - \bar{z}_{t+1}\bar{z}^{T}_{t+1}$ in (B.11) approaches to zero, and the covariance matrix approaches to the following equation (also see in equation (A.12) in Appendix A),

$$\Sigma_{t+1} = \frac{1}{2}U^{-1} + \sum_{j=1}^{4} \theta_{t,j}(\Sigma_t^{-1} + 2F_j)^{-1}. \qquad \text{(B.12)}$$

These equations can be iterated rapidly over time to give an approximation to the time evolution and equilibrium values of $\bar{z}_t$ and Σ_t (We can use (B.11) to do the iteration for the evolution of $\bar{z}_t$ and Σ_t, and both (B.11) and (B.12) can be used to generate equilibrium value of Σ_t).

In the limit $\mu \to 0$, Equation (B.12) approaches the limit

$$\Sigma_{t+1} = \sum_{j=1}^{4} \theta_{t,j}(\Sigma_t^{-1} + 2F_j)^{-1} \qquad \text{(B.13)}$$

from which it can be shown that variance of both the first and second defence evolve towards zero as time t grows (details in Appendix C). If $U \neq 0$, however, it can be shown from (B.12) that the covariance matrix evolves to a non-zero equilibrium. This shows that mutation is necessary in order for the traits to be variable.

3.4 Results

Because of selection, the means of both defences evolve towards the ideal phenotype (this can be shown analytically for the normal approximation— see the iteration equation for the mean (B.10) — and also see numerical results in Figure 3.2 (a), (b)). We are interested in the evolution of the distribution of phenotypes within the population, but in particular in the variance of the phenotypic values. There are five factors that will influence these variances.

1. Mutation

In this model, mutation must be present for phenotypic variance to be maintained — when there is no mutation, the variances of both traits evolve to be zero. This is visible in the numerical results Figure 3.1 (a), (b), and can be shown analytically for the Normal approximation (see Appendix C).

A special case is that when mutation is zero and the first defence is perfectly effective ($\epsilon_1 = 0$), i.e. when the defence will succeed with probability 1 if the trait is at its ideal value $x = a$. As the first defence evolves close to the ideal phenotype, and first defence variance evolves to be zero, the first defence protects all the victims from the enemies so that the second defence is hardly ever tested and evolves very slowly (Figures 3.1 (c)(d), 3.2 (c)(d), 3.3 (b)).

When there is mutation, the variance of both traits evolve to have positive values (Figures 3.1, 3.3). This is proved for the moment closure approximation in Appendix A. Stronger mutation leads to higher variances (Figure 3.3).

2. Order of defence in the sequence

When the first and second defence have the same effectiveness ($\epsilon_1 = \epsilon_2$) and the tolerance range is the same for both defences ($\alpha = \beta$), then the first defence variance is always lower than the second defence variance (i.e., the first defence clusters more closely than the second around its ideal phenotype), no matter what the conditional fitness values are. This is shown in figure 3.6, where var1/var2< 1 (in which var1 stands for the first defence variance, and var2 stands for the second defence variance) along the line $\epsilon_1 = \epsilon_2$; even in figure 3.6 (a), where f_1 is only a little higher than f_2 so that it makes little difference whether the first defence holds or does not, the first defence variance is still a little smaller

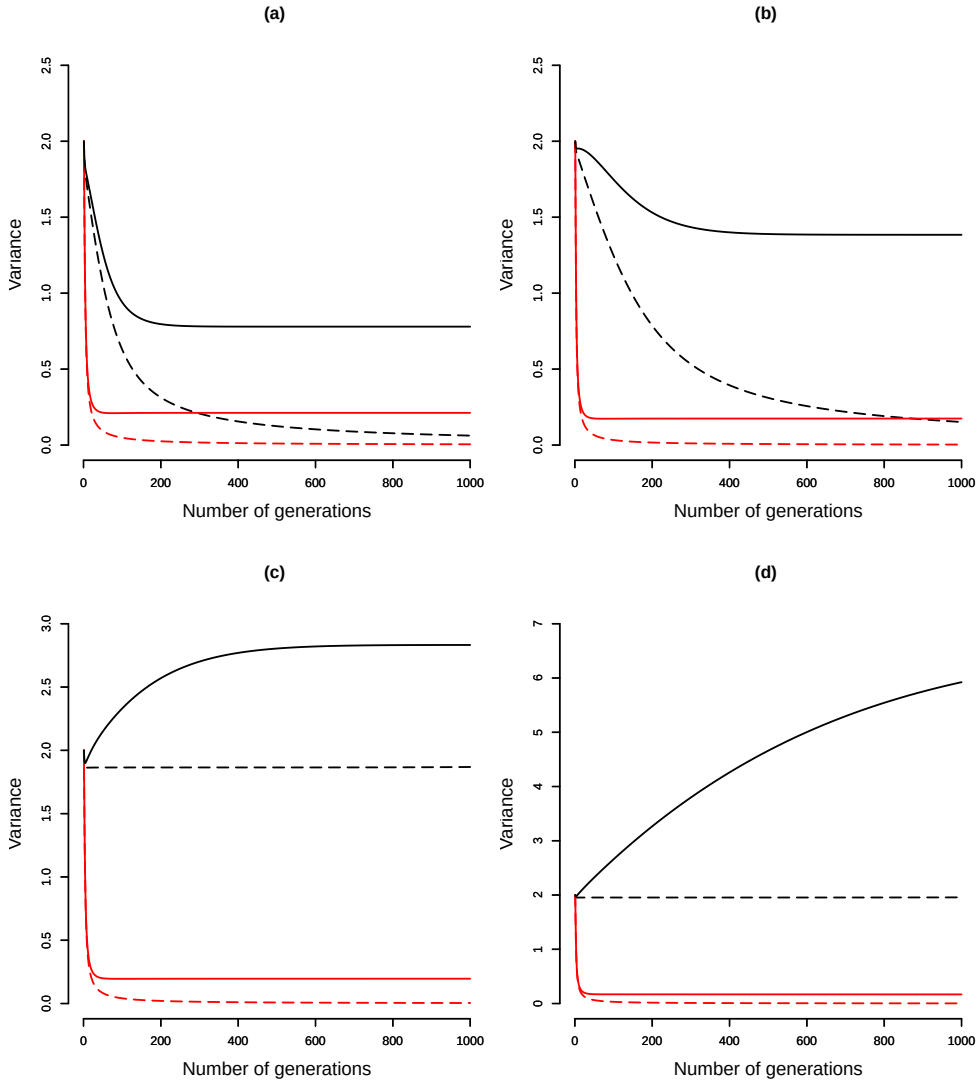


Figure 3.1: The evolution of phenotypic variances, obtained using numerical integration. Dashed lines indicate the case of no mutation, solid lines the case when $\mu = 0.02$. Red lines indicate the variance in the first defense, black lines the variance in the second defense. (a) $\epsilon_1 = 0.1, \epsilon_2 = 0$, (b) $\epsilon_1 = 0.1, \epsilon_2 = 0.9$, (c) $\epsilon_1 = 0, \epsilon_2 = 0$, (d) $\epsilon_1 = 0, \epsilon_2 = 0.9$. Other parameters: $\alpha = 5$, $\beta = 5$, $(f_1, f_2, f_3) = (2, 1, 0.2)$, $(a, b) = (0, 0)$.

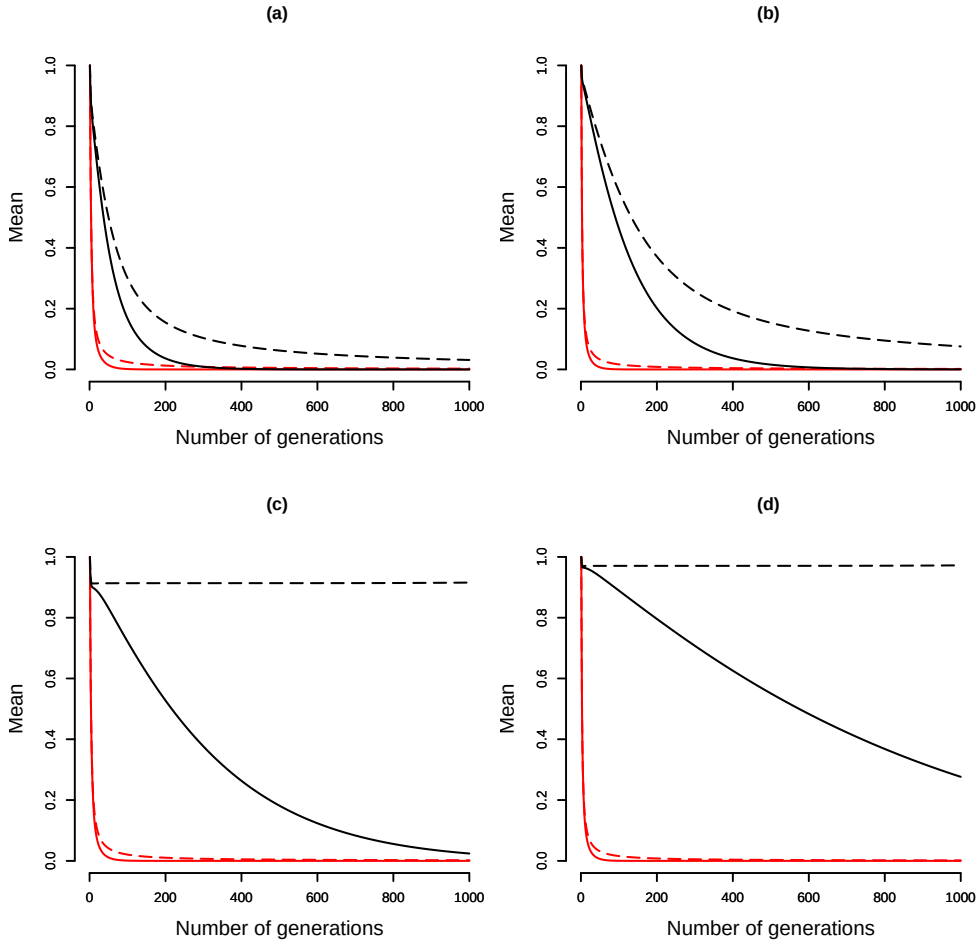


Figure 3.2: The evolution of phenotypic means, obtained from numerical integration. Dashed lines indicate the case of no mutation, solid lines the case when $\mu = 0.02$. Red lines indicate the mean in the first defence, black lines the mean in the second defense. (a) $\epsilon_1 = 0.1, \epsilon_2 = 0$, (b) $\epsilon_1 = 0.1, \epsilon_2 = 0.9$, (c) $\epsilon_1 = 0, \epsilon_2 = 0$, (d) $\epsilon_1 = 0, \epsilon_2 = 0.9$. Other parameters: $\alpha = 5$, $\beta = 5$, $(f_1, f_2, f_3) = (2, 1, 0.2)$, $(a, b) = (0, 0)$.

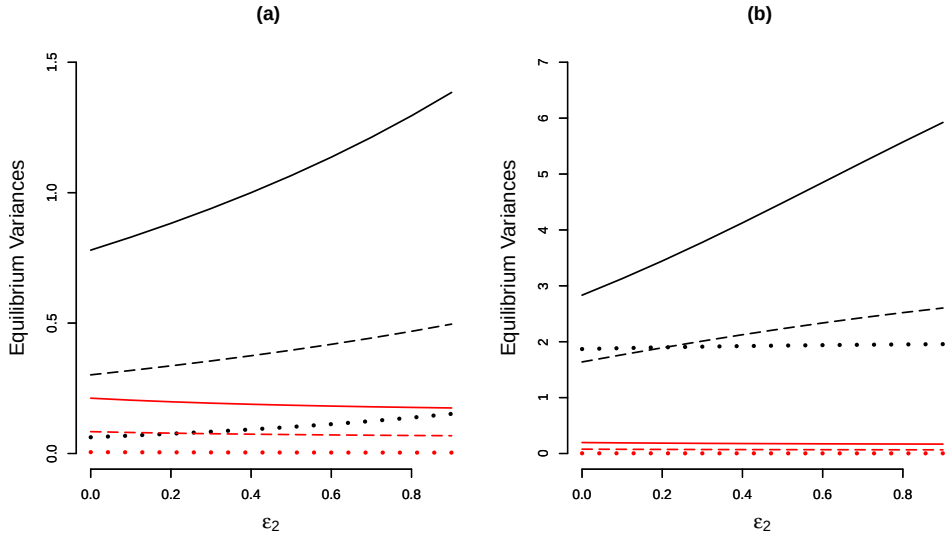


Figure 3.3: The equilibrium values of the variances, obtained from numerical integration. Red lines: first defence; black lines: second defence. Dotted lines: $\mu = 0$; dashed lines: $\mu = 0.01$; solid lines: $\mu = 0.02$. (a) $\epsilon_1 = 0.1$, (b) $\epsilon_1 = 0$. Other parameters: $\alpha = 5$, $\beta = 5$, $(f_1, f_2, f_3) = 2, 1, 0.2$, $(a, b) = (0, 0)$.

than the second defence when $\epsilon_1 = \epsilon_2$.

3. Effectiveness of defences

(1) If the first defence is less effective than the second defence ($\epsilon_1 > \epsilon_2$), then the first defence variance can be larger than the second defence variance (in Figure 3.6, var1/var2> 1 when $\epsilon_1 > \epsilon_2$). The threshold value for the ineffectiveness ϵ_1 of the first defence, above which the first defence has higher variance than the second, depends also on the conditional fitness values (see the contour lines above the red contour line given different fitness values in Figure 3.6 (a-c) described also in "Conditional fitness" below).

(2) When the effectiveness of the first defence increases, the first defence variance decreases and the second defence variances increases. When the effectiveness of the second defence increases, the opposite occurs (see Figure 3.4).

4. Tolerance of phenotypic deviations from the ideal

We refer to the quantities α and β as the "tolerance of phenotypic deviations from the ideal" on the two defensive traits, because they quantify how sensitive the fitness is to deviations from the ideal trait value. However, the variances of the traits do not depend on these quantities in a straightforward way. When the tolerance of deviation from the ideal on a trait is wide, the variance in that trait has a positive relationship with the tolerance as would usually be expected in a mutation-selection balance (Figure 3.5 (a), (e), large values of α or β). However, when the tolerance is narrowed beyond a threshold value, the variance in that trait starts to increase. This is because mutation limits how small the variance in a trait can become, so that as α (for example) decreases more individuals have a maladapted first defence, which as a result is increasingly likely to fail. Since this defence is very likely to fail anyway, its importance in determining the animal's relative fitness actually decreases, and the variance of that trait increases, as α decreases further. (Figure 3.5 (a), (e), small values of α or β). Increasing the ineffectiveness of a defence (ϵ_1 or ϵ_2) makes this effect stronger, so that the positive relationship starts at a smaller value of the tolerance. Since narrowing one defence's tolerance makes it more likely to fail, and therefore makes the other defence more important, the variance in the other defence consequentially decreases (Figure 3.5 (b), (d)). Because the first defence variance and second defence variance can either increase or decrease as the tolerance values change, the ratio of these variances can either increase or decrease (Figure 3.5 (c), (f)).

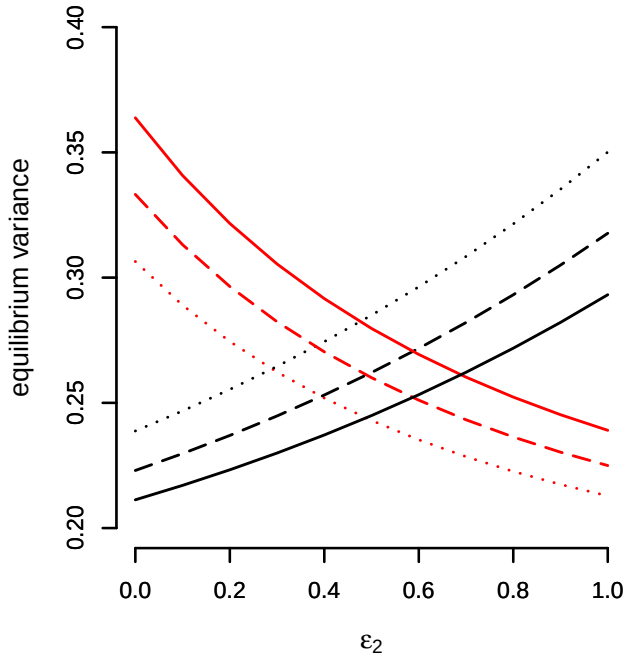


Figure 3.4: The effect of effectiveness on equilibrium variance. Red lines: first defence; black lines: second defence. Dotted lines: $\epsilon_1 = 1.1$; dashed lines: $\epsilon_1 = 1.3$; solid lines: $\epsilon_1 = 1.5$. Other parameters: $\alpha = 5$, $\beta = 5$, $(f_1, f_2, f_3) = (2, 1, 0.2)$, $\mu = 0.02$, $(a, b) = (0, 0)$.

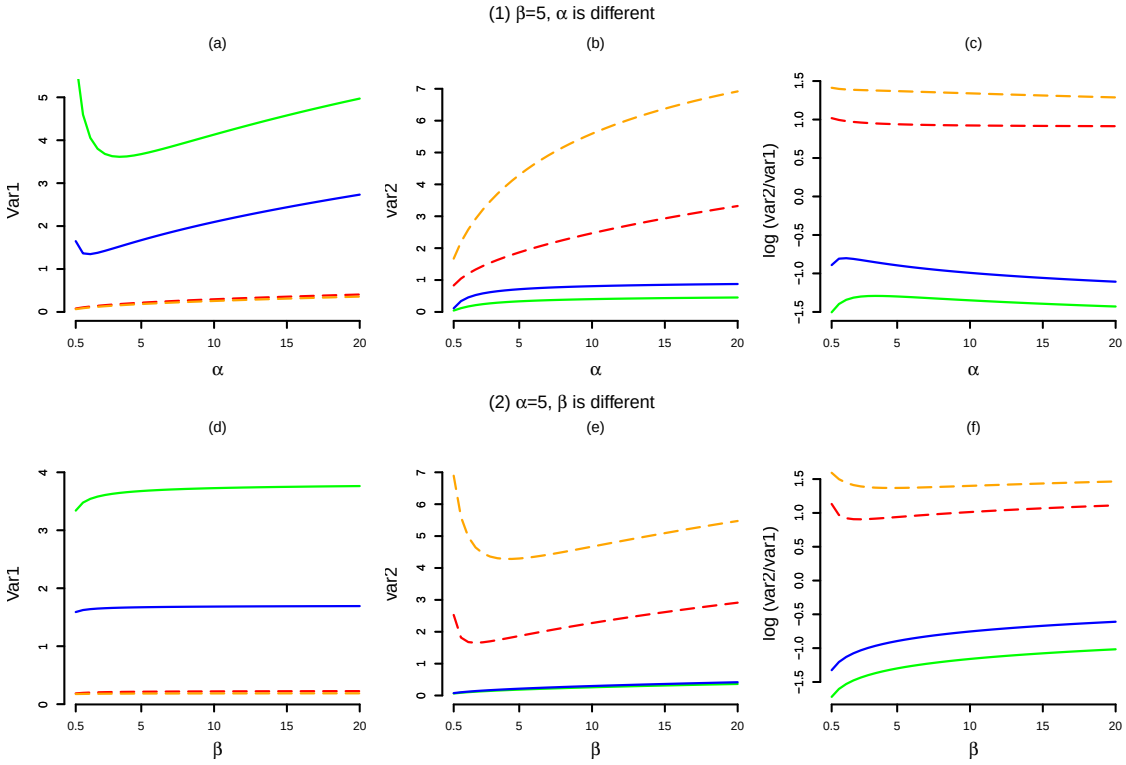


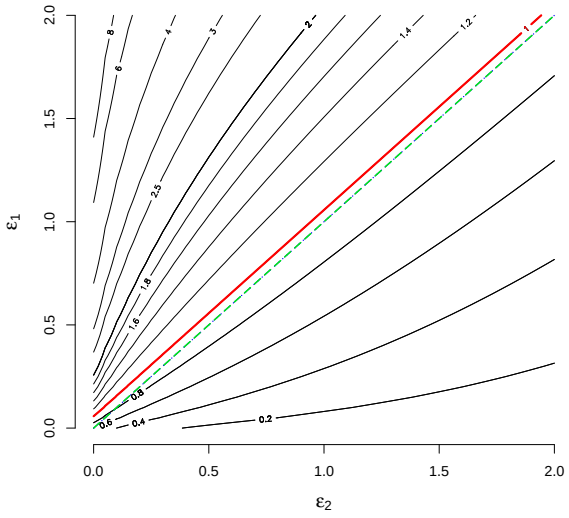
Figure 3.5: The effect of tolerance of phenotypic deviations from the ideal on equilibrium variances. Red line: $\epsilon_1 = 0$, $\epsilon_2 = 0$; yellow line: $\epsilon_1 = 0$, $\epsilon_2 = 0.9$; green line: $\epsilon_1 = 5$, $\epsilon_2 = 0$; black line: $\epsilon_1 = 5$, $\epsilon_2 = 0.9$; In (a,b,c) $\beta = 5$, and in (d,e,f) $\alpha = 5$. Other parameters: $(f_1, f_2, f_3) = (2, 1, 0.2)$, $\mu = 0.02$, $(a, b) = (0, 0)$. These results were obtained by the the Normal Approximation (B.12), which is much faster than the numerical iteration.

5. Conditional fitness

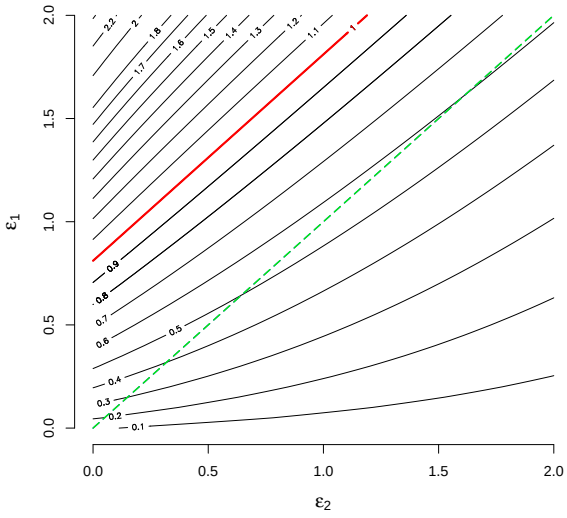
We change the relative values of $\frac{f_1}{f_2}$ (the ratio between the conditional fitness f_1 while the first defence holds and the conditional fitness f_2 while the first defence fails) and $\frac{f_2}{f_3}$ (the ratio between the conditional fitness f_2 while the second defence holds and the conditional fitness f_3 while the second defence fails) to see the relative importance of the first and second defences. When the relative fitness value $\frac{f_1}{f_2}$ increases and $\frac{f_2}{f_3}$ decreases, meaning that the first defence becomes more important, then var1/var2 decreases (given the same ϵ_1 and ϵ_2). This can be seen in Figure 3.6(a), where $\frac{f_1}{f_2}$ is lowest and $\frac{f_2}{f_3}$ is highest, the value of var1/var2 (keeping the same values of (ϵ_1, ϵ_2))is highest; and in Figure 3.6(c) where $\frac{f_1}{f_2}$ is highest and $\frac{f_2}{f_3}$ is lowest, the value of var1/var2 is lowest. As seen in Figure 3.6, the second defence must be much more effective than the first defence ($\epsilon_2 \ll \epsilon_1$, the upper-left side of red solid lines) for the first defence variance to be larger than the second defence variance. Note that here only three typical cases of fitness values are showed (in Figure 3.6(a)(b)(c) respectively) because for the other values (e.g. $f_2 = 0$ which may correspond to that a victim animal is killed when the second defence is breached, or the other values of $f_2 > 0$ which may correspond to that a victim plant is still alive when the second defence is breached), the figures are similar and the relation showed above keep the same.

3.5 Discussion

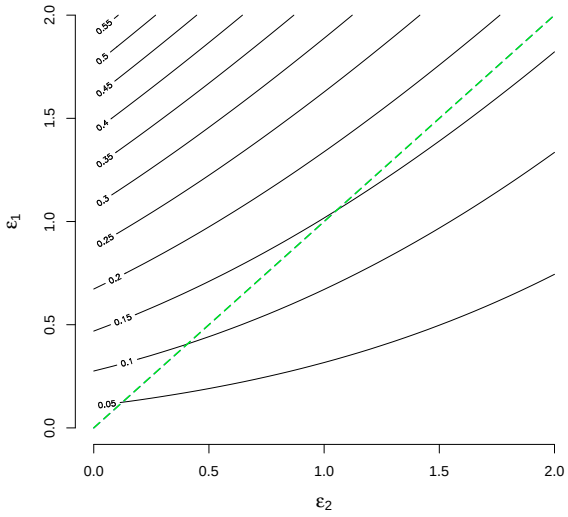
In this chapter we aimed to predict and explain patterns in the variation of anti-predator defences, when those defences are deployed in a predictable sequence. It is well known that defences can be variable in a population, but there is relatively little systematic evaluation of patterns of variation, even though diversifying evolutionary mechanisms are easily identified [22, 23, 32, 33, 34]. It is our contention that the sequential nature of defence may often cause predictable patterns of diversity, allowing testable hypotheses about defence variation. Hence, we built and interrogated a model representing both the selection and mutation mechanisms on the evolution of population distribution of two sequential defences. By using both analytical and numerical methods, we get the evolution processes and equilibrium evolution values of the variances in both defences. We first briefly account for the major determinants of defence variation in our model and subsequently relate its general findings to a wider set of defences and to other theoretical treatments of defence evolution.



(a) $(f_1, f_2, f_3) = (2, 1.9, 0.2)$



(b) $(f_1, f_2, f_3) = (2, 1, 0.2)$



(c) $(f_1, f_2, f_3) = (2, 0.3, 0.2)$

Figure 3.6: Contours of var1/var2 in the (ϵ_1, ϵ_2) plane, for different conditional fitness values. Red solid line: the contour line var1/var2=1 (above which var1/var2$>$ 1, below which var1/var2 $<$ 1) (red line is not visible in (c) as it occurs only when $\epsilon_1 > 2$, which is out off the range of ϵ_1-axis); green dashed line: the line $\epsilon_1 = \epsilon_2$. (a) $(f_1, f_2, f_3) = (2, 1.9, 0.2)$, (b) $(f_1, f_2, f_3) = (2, 1, 0.2)$, (c) $(f_1, f_2, f_3) = (2, 0.3, 0.2)$. Other parameters: $\alpha = 5$, $\beta = 5$, $\mu = 0.02$, $(a, b) = (0, 0)$. These results were obtained by the the Normal Approximation (B.12), which is much faster than the numerical iteration.

3.5.1 Factors predicted to be influential in defence variance

(1) Mutation. Mutation is the reason of why there are defence variances in our model. In the absence of mutation, both defence variances will evolve toward zero, whereas if there is mutation, equilibria of mutation and selection that give variances > 0. Unsurprisingly, the stronger the mutation is, the larger the two defence variances evolve to be.

(2) Order of defence in the sequence. If the first defence and the second defence are as effective as each other, and the tolerance range is the same for both defences, then the first defence distribution evolves to have smaller variance than the second defence distribution (see Results, Figure 3.6). That means the first defence is more closely gathered around the ideal phenotype and therefore has more influence in protecting the victims from being attacked. Hence, the model demonstrates our verbal argument in the introduction: that earlier acting defences can often evolve to lower levels of variation than later acting defences.

(3) Effectiveness of defences. Whether the defences are effective enough in the environment (in the sense of successfully repelling an enemy) is also important to the evolution of population variance. If the first defence in a sequence is not as effective in repelling predators as the second defence, then the force of selection can be felt most strongly on the second defence, with the consequence that it has a lower equilibrium variance than the first defence. This is counter to the intuition in our Introduction, that defences deployed earlier are less variable than those deployed later in sequence, and shows the value of a formal model.

(4) Tolerance of phenotypic deviations from the ideal. We consider that effectiveness of a defence in repelling enemies becomes weaker as the phenotype diverges from the ideal value for the relevant trait. A key measure in this model is therefore how much defensive effectiveness is lost for an incremental deviation from the ideal phenotypic value: in effect the tolerance of the phenotype in relation to its defensive function (α, β). If tolerance of phenotypic deviation is narrow, then even when the phenotype is similar to the ideal phenotype, the defence is likely to fail and be breached. On the contrary, if the tolerance is very wide and permissive, even the phenotype is quite dissimilar to the ideal phenotype, the defence is likely to hold. Both the first and second defence variances will evolve to be high when the tolerance is very narrow or wide. When the tolerance is very wide, the phenotypes

quite different from the ideal phenotypes are effective to protect the victims, then the population variance could evolve to be very large. When the tolerance is very narrow, even the phenotypes are quite similar as the ideal phenotypes are useless in protecting the victims, then it will not be profitable for the phenotypes to evolve to be similar to the ideal phenotypes, so the population variance will also be very wide. An interesting result pertains now if the first defence is subject to narrow tolerance and the second defence to wider tolerance. Here the first defence can be of little use, and contributes little to prey survival, hence mutation accumulates and the phenotype becomes variable. Variation in the second defence however is fundamental to prey survival, hence the model predicts a lower equilibrium value for mutation (Figure 3.5). This gives us an additional scenario in which the first defence may evolve to a higher level of variation than the second.

(5) Conditional Fitness. In our model, the relative importance of whether the first defence holds to whether the second defence holds are described by the relative conditional fitness values. When the relative conditional fitness value $\frac{f_1}{f_2}$ increases (which means that the importance of holding the first defence increases) and the relative conditional fitness value $\frac{f_2}{f_3}$ decreases (which means that the importance of holding the second defence increases), then the ratio between the first defence variance and the second defence variance decreases. The contrary is true when the conditions are reversed.

3.5.2 Application to biological and other contexts

Sequentially-layered defences are very common in biological and other contexts. Many plants and animals present their enemies with layered defences. John Endler for example [28] argued that an attack by a predator on its (animal) prey is typically composed of a sequence of six stages: (i) encounter (spatial proximity), (ii) detection, (iii) identification, (iv) approach, (v) subjugation and ultimately (vi) consumption. At each stage in this sequence the prey organism can put up one or more lines of defence with the aim of preventing, interrupting and stopping the attack. An animal prey may for example hide (to prevent encounter, i, and detection, ii), use masquerade and cryptic colouration (to prevent detection and identification, ii, iii), perhaps form aggressive defensive groups (to prevent approach, iv). They may alternatively have a startle display or use vigilance and rapid escape behaviours (to prevent approach, iv). They may violently retaliate (to prevent subjugation, v.) perhaps using stings, spines or bites and/or deploy irritating or

toxic chemicals (to prevent subjugation and consumption, v, vi). At each stage in the sequence Endler identified, one or more defences could be deployed by a prey animal, and they could often operate sequentially, some defences typically used only if earlier-acting defences have failed to stop the predation event. Here we have simplified to two layers, but the model could be extended to larger set of defences. An important point is, however, that we expect sequentially acting defences to be very common in organismal defence, hence our model has generality.

One very general result is greater variation in later-acting defences. There is some evidence supportive of a key feature of the model, that later-acting defences are used less often than earlier defences, and thus contribute less to fitness. A meta-analysis of studies of plant-herbivore interactions shows that variation in earlier-acting defences (physical, morphological, physiology, chemical defences) in plants is better in predicting herbivores' damage than later defences (toxic secondary metabolites; [35, 36]). A number of authors have remarked on the high levels of variability in defensive toxins (see review in ref. [23]), but we do not know of studies that measure the variability of sets of defences and relate these to their use in a sequence. We suggest that this is an interesting area for valuable empirical research.

We note that anti-pathogen systems (skin, immune responses) are also usually layered in their organisation, hence the model could be elaborated to consider these kinds of defensive systems. There are also interesting parallels between the organisation of biological and human military defences. Both concern protection of valuable yet vulnerable targets, seeking optimal deployment of costly defensive "assets". A relevant military tactic is "layered defence" in which sets of defensive resources, such as inter-ballistic missiles, are deployed in sequence; when a first line of defence fails against an incoming threat a second line of defence activates to minimise further risk, and after that perhaps a third or fourth defence, and so on. In the military theory literature, layered defence has been described and modelled by Wilkening [37]. We suggest that it might be an interesting question to determine whether, in military contexts and perhaps cyber-security, later-acting defences are more variable in their form and effectiveness than earlier-acting defences.

3.5.3 Developments of the model

We would draw the reader's attention to some key assumptions in the model. First, we assume that there is an optimal value for each defence, and any deviation from that is punished by reduced efficiency in repelling enemies. This assumption does simplify implementation, giving us a clear set of results, but it does bring some limitations. On the one hand, this assumption may fit morphological defences well - for example defensive spines may need to be the right size to repel certain enemies. It does not represent some kinds of chemical defence as well however. Here concentrations that are too low may lead to reduced efficiency, but higher and higher values probably become more effective at anti-predator defence, albeit in a saturating manner, not less. In this case the model would have to be modified to incorporate this asymmetry in defensive benefit. An interesting question is whether the distribution of naturally occurring defensive toxins is asymmetric in this manner. Secondly, we assumed that once each defence is breached, it cannot be healed (e.g. the spines of golden barrel cactus *Echinocactus grusonii* once moved from areole cannot grow back), in comparison to that for some organisms, defences can replenish when damaged (e.g. the claw of Florida stone crab, *Menippe mercenaria* can grow back when broken with the diaphragm at the claw joint intact). Also for the case such as different parts of plants maybe attacked independently, defences can be breached several times in these different parts. Since our model is based on an average fitness, it can describe qualitatively the dependence of the relative variances on fitness costs and tolerances in the above cases, but it would be good to be further modified in the case of multiple successive breaches of each defence (where the organism heals between each attack) and may also for the case when different parts of plants are breached independently. Finally, we have deliberately excluded costs of defences in the model, in part to keep the structure simple and predictions tractable, but there is profitable scope for including costs in a more complex development. We note that studies of prey defence can not always identify measurable costs to defence in any case (Zvereva & Kozlov [38]).

3.6 Conclusion

We aimed to explore the patterns of the defence variations when defences are deployed in sequence. We built a model with two sequential defences, and use both selection and

mutation as the evolutionary mechanisms on the evolution of population distribution of the two defences. Through both analytical and numerical methods, we found that typically the earlier defence has lower variance than the later defence, which means that the earlier defence phenotypes are more closely accumulated around the ideal phenotype than the later defence phenotypes. This matches with intuition and some research that the earlier defences have higher probability in use and therefore probably have higher anti-predator effect. Besides, our formal model also gives a broader explanation that when the first defence is less effective in repelling the predators, or the first defence is less tolerant of phenotypic deviations from the ideal, then the first defence could evolve to have higher variance than the second defence. Sequential defences are widely seen in different defence systems, therefore our model might be predictive in a wide range of areas. Since the empirical research of sequential defence variances is rare, related research could be valuable.

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3.7 Appendices

3.7.1 Appendix A

To get the population distribution at the next generation $N_{t+1}(x_{t+1}, y_{t+1})$ (equation (B.9)) we first write the fitness function in matrix form. From (B.1) and (B.2), the fitness function (B.3) can be written as

$$\Phi(x_t, y_t) = f_3 + (f_1 - f_3)e^{-\epsilon_1 - \frac{(x_t-a)^2}{\alpha}} + (f_2 - f_3)e^{-\epsilon_2 - \frac{(y_t-b)^2}{\beta}} + (f_3 - f_2)e^{-\epsilon_1 - \frac{(x_t-a)^2}{\alpha}} e^{-\epsilon_2 - \frac{(y_t-b)^2}{\beta}}$$

$$= \sum_{j=1}^{4} \gamma_j \exp\Big(- (z_t - \hat{a})^T F_j (z_t - \hat{a})\Big) \tag{A.1}$$

where,

$$\gamma_1 = f_3, \quad \gamma_2 = (f_1 - f_3)e^{-\epsilon_1}, \quad \gamma_3 = (f_2 - f_3)e^{-\epsilon_2}, \quad \gamma_4 = (f_3 - f_2)e^{-(\epsilon_1+\epsilon_2)},$$

$$F_1 = \begin{pmatrix} 0 & 0 \\ 0 & 0 \end{pmatrix}, \quad F_2 = \begin{pmatrix} \frac{1}{\alpha} & 0 \\ 0 & 0 \end{pmatrix}, \quad F_3 = \begin{pmatrix} 0 & 0 \\ 0 & \frac{1}{\beta} \end{pmatrix}, \quad F_4 = \begin{pmatrix} \frac{1}{\alpha} & 0 \\ 0 & \frac{1}{\beta} \end{pmatrix}$$

$$z_t - \hat{a} = \begin{pmatrix} x_t - a \\ y_t - b \end{pmatrix}, \quad \hat{a} = \begin{pmatrix} a \\ b \end{pmatrix}$$

The mutation function (B.6) can be written as

$$M(x_t, y_t; x_{t+1}, y_{t+1}) = \frac{1}{\pi\mu} \exp\Big(-(z_t - z_{t+1})^T U (z_t - z_{t+1}) \Big) \tag{A.2}$$

where $z_t - z_{t+1} = \begin{pmatrix} x_t - x_{t+1} \\ y_t - y_{t+1} \end{pmatrix}$, $U = \begin{pmatrix} \frac{1}{\mu} & 0 \\ 0 & \frac{1}{\mu} \end{pmatrix}$.

Then from (B.8), (A.1) and (A.2), the population distribution density iteration function (B.5) is as follows.

$$N_{t+1}(x_{t+1}, y_{t+1}) = \frac{n_t\sqrt{|W_t|}}{\pi^2\mu} \int_{-\infty}^{+\infty} \int_{-\infty}^{+\infty} \sum_{j=1}^{4} \gamma_j \exp\Big(-(z_t - \bar{z}_t)^T W_t (z_t - \bar{z}_t) - (z_t - \hat{a})^T F_j (z_t - \hat{a})$$

$$-(z_t - z_{t+1})^T U (z_t - z_{t+1}) \Big) dx_t dy_t \tag{A.3}$$

Since z_t is a normal distributed vector, we collect terms for z_t, let $K_{t,j} = W_t + F_j + U$, and complete the square for z_t:

$$N_{t+1}(x_{t+1}, y_{t+1}) = \frac{n_t\sqrt{|W_t|}}{\pi^2\mu} \int_{-\infty}^{+\infty} \int_{-\infty}^{+\infty} \sum_{j=1}^{4} \gamma_j \exp\Big(-z_t^T K_{t,j} z_t + 2(W_t \bar{z}_t + F_j \hat{a} + U z_{t+1})^T z_t$$

$$-(\bar{z}_t^T W_t \bar{z}_t + \hat{a}^T F_j \hat{a} + z_{t+1}^T U z_{t+1})\Big) dx_t dy_t$$

$$= \frac{n_t\sqrt{|W_t|}}{\pi^2\mu} \int_{-\infty}^{+\infty}\int_{-\infty}^{+\infty} \sum_{j=1}^{4} \gamma_j \exp\Big(-(z_t - K_{t,j}^{-1}(W_t\bar{z}_t + F_j\hat{a} + Uz_{t+1}))^T K_{t,j}(z_t - K_{t,j}^{-1}(W_t\bar{z}_t + F_j\hat{a} + Uz_{t+1}))$$

$$+(W_t\bar{z}_t + F_j\hat{a} + Uz_{t+1})^T K_{t,j}^{-1}(W_t\bar{z}_t + F_j\hat{a} + Uz_{t+1}) - (\bar{z}_t^T W_t \bar{z}_t + \hat{a}^T F_j \hat{a} + z_{t+1}^T U z_{t+1})\Big) dx_t dy_t$$

From the fact that the integral of normal density function equals 1, the above is equivalent to

$$N_{t+1}(x_{t+1}, y_{t+1}) = \frac{n_t\sqrt{|W_t|}}{\pi^2\mu} \sum_{j=1}^{4} \gamma_j \frac{\pi}{\sqrt{|K_{t,j}|}} \exp\Big((W_t\bar{z}_t + F_j\hat{a} + Uz_{t+1})^T K_{t,j}^{-1}(W_t\bar{z}_t + F_j\hat{a} + Uz_{t+1})$$

$$-(\bar{z}_t^T W_t \bar{z}_t + \hat{a}^T F_j \hat{a} + z_{t+1}^T U z_{t+1})\Big) \tag{A.4}$$

Since z_{t+1} is the next generation defence phenotype vector, if we collect terms for z_{t+1}, given that $U = \begin{pmatrix} \frac{1}{\mu} & 0 \\ 0 & \frac{1}{\mu} \end{pmatrix}$, we have

$$N_{t+1}(x_{t+1}, y_{t+1}) = \frac{n_t}{\pi\mu}\sqrt{|W_t|} \sum_{j=1}^{4} \frac{\gamma_j}{\sqrt{|K_{t,j}|}} \exp\Big(-\frac{1}{\mu} z_{t+1}^T (I - (\mu K_{t,j})^{-1}) z_{t+1}$$

$$+2\frac{1}{\mu}(W_t\bar{z}_t + F_j\hat{a})^T K_{t,j}^{-1} z_{t+1} + (W_t\bar{z}_t + F_j\hat{a})^T K_{t,j}^{-1}(W_t\bar{z}_t + F_j\hat{a}) - \bar{z}_t^T W_t \bar{z}_t - \hat{a}^T F_j \hat{a}\Big)$$

To complete a square, the above is equivalent to

$$N_{t+1}(x_{t+1}, y_{t+1}) = \frac{n_t}{\pi\mu}\sqrt{|W_t|} \sum_{j=1}^{4} \frac{\gamma_j}{\sqrt{|K_{t,j}|}} \exp\Big(-\frac{1}{\mu}(z_{t+1} - z'_{t+1,j})^T (I - (\mu K_{t,j})^{-1})(z_{t+1} - z'_{t+1,j})$$

$$+\frac{1}{\mu} z'^T_{t+1,j}(I - (\mu K_{t,j})^{-1}) z'_{t+1,j} + (W_t\bar{z}_t + F_j\hat{a})^T K_{t,j}^{-1}(W_t\bar{z}_t + F_j\hat{a}) - \bar{z}_t^T W_t \bar{z}_t - \hat{a}^T F_j \hat{a}\Big)$$

where $z'_{t+1,j} = (I - (\mu K_{t,j})^{-1})^{-1} K_{t,j}^{-1}(W_t\bar{z}_t + F_j\hat{a}) = (K_{t,j} - \frac{1}{\mu} I)^{-1}(W_t\bar{z}_t + F_j\hat{a}) = (W_t +$

$F_j)^{-1}(W_t\bar{z}_t+F_j\hat{a})$. If we put the expression of $z'_{t+1,j}$ into the second term in the exponential bracket, and combine the second term with the third term, we have

$$N_{t+1}(x_{t+1},y_{t+1}) = \frac{n_t}{\pi\mu}\sqrt{|W_t|}\sum_{j=1}^{4}\frac{\gamma_j}{\sqrt{|K_{t,j}|}}\exp\Big(-\frac{1}{\mu}(z_{t+1}-z'_{t+1,j})^T(I-(\mu K_{t,j})^{-1})(z_{t+1}-z'_{t+1,j})$$

$$+\frac{1}{\mu}(W_t\bar{z}_t+F_j\hat{a})^T\big((K_{t,j}-\frac{1}{\mu}I)^{-1}K_{t,j}^{-1}+\mu K_{t,j}^{-1}\big)(W_t\bar{z}_t+F_j\hat{a})-\bar{z}_t^TW_t\bar{z}_t-\hat{a}^TF_j\hat{a}\Big)$$

Since $K_{t,j}=W_t+F_j+U$, we have

$$N_{t+1}(x_{t+1},y_{t+1}) = \frac{n_t}{\pi\mu}\sqrt{|W_t|}\sum_{j=1}^{4}\frac{\gamma_j}{\sqrt{|K_{t,j}|}}\exp\Big(-\frac{1}{\mu}(z_{t+1}-z'_{t+1,j})^T(I-(\mu K_{t,j})^{-1})(z_{t+1}-z'_{t+1,j})$$

$$+\frac{1}{\mu}(W_t\bar{z}_t+F_j\hat{a})^T\big((W_t+F_j)^{-1}K_{t,j}^{-1}+\mu K_{t,j}^{-1}\big)(W_t\bar{z}_t+F_j\hat{a})-\bar{z}_t^TW_t\bar{z}_t-\hat{a}^TF_j\hat{a}\Big)$$

If we let $s_{t,j}=\exp\Big(\frac{1}{\mu}(W_t\bar{z}_t+F_j\hat{a})^T\Big((W_t+F_j)^{-1}+\mu I\Big)K_{t,j}^{-1}(W_t\bar{z}_t+F_j\hat{a})-\bar{z}_t^TW_t\bar{z}_t-\hat{a}^TF_j\hat{a}\Big)$, then

$$N_{t+1}(x_{t+1},y_{t+1}) = \frac{n_t}{\pi\mu}\sqrt{|W_t|}\sum_{j=1}^{4}\frac{\gamma_j}{\sqrt{|K_{t,j}|}}s_{t,j}\exp\Big(-\frac{1}{\mu}(z_{t+1}-z'_{t+1,j})^T(I-(\mu K_{t,j})^{-1})(z_{t+1}-z'_{t+1,j})\Big)$$

Then we can write it into the normal distribution form:

$$N_{t+1}(x_{t+1},y_{t+1}) = n_t\sqrt{|W_t|}\sum_{j=1}^{4}\frac{\gamma_j s_{t,j}}{\sqrt{|W_t+F_j|}}$$

$$\frac{\sqrt{|(I-(\mu K_{t,j})^{-1})|}}{\pi\mu}\exp\Big(-\frac{1}{\mu}(z_{t+1}-z'_{t+1,j})^T(I-(\mu K_{t,j})^{-1})(z_{t+1}-z'_{t+1,j})\Big)$$

This is a combination of four normal distribution functions, with mean $z'_{t+1,j}=(W_t+$

$F_j)^{-1}(W_t\bar{z}_t + F_j\hat{a})$, and variance

$$\Sigma_{t+1,j} = \frac{\mu}{2}(I - (\mu K_{t,j})^{-1})^{-1} = \frac{\mu}{2}(K_{t,j} - \frac{1}{\mu}I)^{-1}K_{t,j} = \frac{\mu}{2}(W_t + F_j)^{-1}(W_t + F_j + U)$$

$$= \frac{\mu}{2}I + \frac{1}{2}(W_t + F_j)^{-1} = \frac{1}{2}\Big(U^{-1} + (W_t + F_j)^{-1}\Big) = \frac{1}{2}\Big(U^{-1} + (\frac{1}{2}\Sigma_t^{-1} + F_j)^{-1}\Big)$$

$$= \frac{1}{2}U^{-1} + (\Sigma_t^{-1} + 2F_j)^{-1} \qquad j = 1, 2, 3, 4 \tag{A.5}$$

So the next generation population function can be written as

$$N_{t+1}(x_{t+1}, y_{t+1}) = n_t\sqrt{|W_t|}\sum_{j=1}^{4}\frac{\gamma_j s_{t,j}}{\sqrt{|W_t + F_j|}}\frac{1}{2\pi\sqrt{|\Sigma_{t+1,j}|}}\exp\Big(-\frac{1}{2}(z_{t+1} - z'_{t+1,j})^T\Sigma_{t+1,j}^{-1}(z_{t+1} - z'_{t+1,j})\Big)$$

Let $\Theta_t = \sum_{j=1}^{4}\frac{\gamma_j s_{t,j}}{\sqrt{|W_t+F_j|}}$, and $\theta_{t,j} = \frac{\gamma_j s_{t,j}}{\Theta_t\sqrt{|W_t+F_j|}}$ $(j = 1, 2, 3, 4)$ then $\sum_{j=1}^{4}\theta_{t,j} = 1$. Then the above is equivalent to

$$N_{t+1}(x_{t+1}, y_{t+1}) = n_t\sqrt{|W_t|}\Theta_t\sum_{j=1}^{4}\theta_{t,j}\frac{1}{2\pi\sqrt{|\Sigma_{t+1,j}|}}\exp\Big(-\frac{1}{2}(z_{t+1} - z'_{t+1,j})^T\Sigma_{t+1,j}^{-1}(z_{t+1} - z'_{t+1,j})\Big)$$

Therefore, the **probability density function** in the next generation can be written as

$$f(x_{t+1}, y_{t+1}) = N_{t+1}(x_{t+1}, y_{t+1}) / \int_{-\infty}^{+\infty}\int_{-\infty}^{+\infty} N_{t+1}(x_{t+1}, y_{t+1})dz_{t+1}$$

$$= \sum_{j=1}^{4}\theta_{t,j}\frac{1}{2\pi\sqrt{|\Sigma_{t+1,j}|}}\exp\Big(-\frac{1}{2}(z_{t+1} - z'_{t+1,j})^T\Sigma_{t+1,j}^{-1}(z_{t+1} - z'_{t+1,j})\Big) \tag{A.6}$$

$$= \sum_{j=1}^{4}\theta_{t,j}f_j(x_{t+1}, y_{t+1})$$

Therefore the population distribution probability density function in the $(t+1)$-th generation is written as a combination of four normal probability density functions

$$z'_{t+1,j} = (W_t + F_j)^{-1}(W_t \bar{z}_t + F_j \hat{a}) \tag{A.7}$$

and covariance matrix

$$\Sigma_{t+1,j} = \frac{1}{2}U^{-1} + (\Sigma_t^{-1} + 2F_j)^{-1} \qquad j = 1, 2, 3, 4 \tag{A.8}$$

Note that the integral of $f(x_{t+1}, y_{t+1})$ in respect of (x_{t+1}, y_{t+1}) equals 1, which is the property of the probability density function. We use $E_{N_j}(\cdot)$ to denote the expectation of each of the four corresponding normal population distribution function.

Therefore the **mean in the next generation** is

$$\begin{aligned}
\bar{z}_{t+1} = E(z_{t+1}) &= \int_{-\infty}^{+\infty}\int_{-\infty}^{+\infty} z_{t+1} f(x_{t+1}, y_{t+1}) dz_{t+1} \\
&= \sum_{j=1}^{4} \theta_{t,j} \int_{-\infty}^{+\infty}\int_{-\infty}^{+\infty} z_{t+1} f_i(x_{t+1}, y_{t+1}) dz_{t+1} \\
&= \sum_{j=1}^{4} \theta_{t,j} E_{N_j}(z_{t+1}) \\
&= \sum_{j=1}^{4} \theta_{t,j} z'_{t+1,j} \\
&= \sum_{j=1}^{4} \theta_{t,j} (W_t + F_j)^{-1}(W_t \bar{z}_t + F_j \hat{a})
\end{aligned} \tag{A.9}$$

This is the iteration equations for the mean between generations.

The above also equals

$$\bar{z}_{t+1} = \sum_{j=1}^{4} \theta_{t,j}(W_t + F_j)^{-1} W_t \bar{z}_t + \sum_{j=1}^{4} \theta_{t,j}(W_t + F_j)^{-1} F_j \hat{a} \tag{A.10}$$

The above is a first-order difference equation. Since $\sum_{j=1}^{4} \theta_{t,j}(W_t + F_j)^{-1} W_t + \sum_{j=1}^{4} \theta_{t,j}(W_t + F_j)^{-1} F_j = I$, the mean $\bar{z}_{t+1}$ will gradually approach to the equilibrium –the ideal phenotype $\hat{a}$ as t increases. This equilibrium value can be got by letting both $\bar{z}_{t+1}$ and $\bar{z}_t$ in the above equation equal z_T and solve the equation, we will have that $z_T = \hat{a}$.

For variance, we have the **covariance matrix in the next generation**

$$\Sigma_{t+1} = E \begin{pmatrix} x_{t+1}^2 & x_{t+1}y_{t+1} \\ x_{t+1}y_{t+1} & y_{t+1}^2 \end{pmatrix} - E \begin{pmatrix} x_{t+1} \\ y_{t+1} \end{pmatrix} E \begin{pmatrix} x_{t+1} & y_{t+1} \end{pmatrix}$$

$$= \int_{-\infty}^{+\infty} \int_{-\infty}^{+\infty} \begin{pmatrix} x_{t+1}^2 & x_{t+1}y_{t+1} \\ x_{t+1}y_{t+1} & y_{t+1}^2 \end{pmatrix} \cdot \sum_{j=1}^{4} \theta_{t,j} f_i(x_{t+1}, y_{t+1}) dz_{t+1} - \bar{z}_{t+1}\bar{z}_{t+1}^T$$

$$= \sum_{j=1}^{4} \theta_{t,j} \cdot \begin{pmatrix} E_{N_j}(x_{t+1}^2) & E_{N_j}(x_{t+1}y_{t+1}) \\ E_{N_j}(x_{t+1}y_{t+1}) & E_{N_j}(y_{t+1}^2) \end{pmatrix} - \bar{z}_{t+1}\bar{z}_{t+1}^T$$

$$= \sum_{j=1}^{4} \theta_{t,j} \cdot \begin{pmatrix} Var_{N_j}(x_{t+1}) + E_{N_j}(x_{t+1})^2 & Cov_{N_j}(x_{t+1}, y_{t+1}) + E_{N_j}(x_{t+1})E_{N_j}(y_{t+1}) \\ Cov_{N_j}(x_{t+1}, y_{t+1}) + E_{N_j}(x_{t+1})E_{N_j}(y_{t+1}) & Var_{N_j}(y_{t+1}) + E_{N_j}(y_{t+1})^2 \end{pmatrix}$$

$$- \bar{z}_{t+1}\bar{z}_{t+1}^T$$

$$= \sum_{j=1}^{4} \theta_{t,j} \cdot \Sigma_{t+1,j} + \sum_{j=1}^{4} \theta_{t,j} \cdot z'_{t+1,j} z'^T_{t+1,j} - \bar{z}_{t+1}\bar{z}_{t+1}^T$$

$$= \sum_{j=1}^{4} \theta_{t,j} \Big(\frac{1}{2} U^{-1} + (\Sigma_t^{-1} + 2F_j)^{-1} \Big) + \sum_{j=1}^{4} \theta_{t,j} \cdot z'_{t+1,j} z'^T_{t+1,j} - \bar{z}_{t+1}\bar{z}_{t+1}^T$$

$$= \frac{1}{2} U^{-1} + \sum_{j=1}^{4} \theta_{t,j} (\Sigma_t^{-1} + 2F_j)^{-1} + \sum_{j=1}^{4} \theta_{t,j} \cdot z'_{t+1,j} z'^T_{t+1,j} - \bar{z}_{t+1}\bar{z}_{t+1}^T \tag{A.11}$$

where $z'_{t+1,j}$ is the j-th mean shown in (A.7).

Now we can use the variance iteration equations from (A.11) to get the evolution of variance across generations. As $\bar{z}_t$ approaches to the ideal phenotype $\hat{a}$ as $t \to +\infty$, so does $\bar{z}_{t+1}$ and $z'_{t+1,j} = (W_t + F_j)^{-1}(W_t\bar{z}_t + F_j\hat{a})$ $(j = 1, 2, 3, 4)$ also approaches to the ideal phenotype $\hat{a}$. Therefore the term $\sum_{j=1}^{4} \theta_{t,j} \cdot z'_{t+1,j} z'^{T}_{t+1,j} - \bar{z}_{t+1}\bar{z}^{T}_{t+1}$ in (A.11) approaches to zero, so covariance matrix (A.11) approaches to the following equation as time grows.

$$\Sigma_{t+1} = \frac{1}{2}U^{-1} + \sum_{j=1}^{4} \theta_{t,j}(\Sigma_t^{-1} + 2F_j)^{-1} \tag{A.12}$$

which is larger than $\frac{1}{2}U^{-1}$, since $\frac{1}{2}\sum_{j=1}^{4} \theta_{t,j}(\Sigma_t^{-1} + F_j)^{-1}$ is larger than zero, so as t goes to infinity, the equilibrium value of Σ_t will be larger than $\frac{1}{2}U^{-1}$. Therefore, if there is mutation $(U > 0)$, variances of both defences will be positive (larger than $\frac{\mu}{2}$).

3.7.2 Appendix B

The scale of the variance in defence phenotypes is set by the parameters μ, α, and β. In this appendix, we show that if we increase these three parameters by a common factor, the equilibrium value of v_t and w_t will change by the same factor.

Let $P_t(x, y)$ denote the probability distribution of traits at time t, where

$$P_t(x, y) = \frac{N_t(x, y)}{\int\int N_t(x, y)dx\, dy}.$$

The integration limits are from $-\infty$ to ∞, and are suppressed throughout this section for brevity. From eqn. (B.5), the dynamics of P_t is determined by

$$\begin{aligned} P_{t+1}(x, y) &= \frac{N_{t+1}(x, y)}{\int\int N_{t+1}(x, y)dx\, dy} \\ &= \frac{\int\int P_t(x', y')\Phi(x', y')M(x', y', x, y)dx'\, dy'}{\int\int P_t(x', y')\Phi(x', y')dx'\, dy'}, \end{aligned}$$

where we have used the fact that $\int\int M(x', y', x, y)dx\, dy = 1$. Over time, P_t will approach

an equilibrium $P_*(x,t) = \lim_{t\to\infty} P_t(x,t)$, where

$$P_*(x,y) = \frac{\int\int P_*(x',y')\Phi(x',y')M(x',y',x,y)dx'\,dy'}{\int\int P_*(x',y')\Phi(x',y')dx'\,dy'}. \tag{B.1}$$

From Eqns. (B.1–B.3) and (B.6), Φ and M can be written in the form

$$\Phi(x,y) = \tilde{\Phi}\left(\frac{x-a}{\alpha^{1/2}}, \frac{y-b}{\beta^{1/2}}\right) \tag{B.2}$$

$$M(x',y',x,y) = \frac{1}{\mu}\tilde{M}\left(\frac{x-x'}{\mu^{1/2}}, \frac{y-y'}{\mu^{1/2}}\right), \tag{B.3}$$

where

$$\tilde{\Phi}(u,v) = f_1 e^{-\epsilon_1-u^2} + \left(1-e^{-\epsilon_1-u^2}\right)\left(\left(f_2-f_3\right)e^{-\epsilon_2-v^2}+f_3\right) \tag{B.4}$$

$$\tilde{M}(u,v) = \frac{1}{\pi}e^{-u^2-v^2}. \tag{B.5}$$

We now substitute eqns. (B.2) and (B.3) into Eqn. (B.1), make the change of variables

$$\xi = \frac{x-a}{\mu^{1/2}}$$
$$\xi' = \frac{x'-a}{\mu^{1/2}}$$
$$\eta = \frac{y-b}{\mu^{1/2}}$$
$$\eta' = \frac{y'-b}{\mu^{1/2}},$$

and further define (without loss of generality)

$$P_*(x,y) = \frac{1}{\mu}\tilde{P}_*(\xi,\eta), \tag{B.6}$$

to give

$$\tilde{P}_*(\xi,\eta) = \frac{\int\int \tilde{P}_*(\xi',\eta')\tilde{\Phi}\left(\left(\frac{\mu}{\alpha}\right)^{1/2}\xi', \left(\frac{\mu}{\beta}\right)^{1/2}\eta'\right)\tilde{M}(\xi-\xi',\eta-\eta')d\xi'\,d\eta'}{\int\int \tilde{P}_*(\xi',\eta')\tilde{\Phi}\left(\left(\frac{\mu}{\alpha}\right)^{1/2}\xi', \left(\frac{\mu}{\beta}\right)^{1/2}\eta'\right)d\xi'\,d\eta'}. \tag{B.7}$$

Note that, from eqns. (B.4,B.5), neither $\tilde{\Phi}$ nor $\tilde{M}$ have any explicit dependence on μ, α, or β so these parameters only enter into eqn. (B.7) through the ratios $\frac{\mu}{\alpha}$ and $\frac{\mu}{\beta}$ in the arguments to $\tilde{\Phi}$. This means that the solution $\tilde{P}_*(\xi,\eta)$ to eqn. (B.7) can be written in the form

$$\tilde{P}_*(\xi,\eta) = p\left(\xi,\eta,\frac{\mu}{\alpha},\frac{\mu}{\beta}\right),$$

where p does not depend explicity on μ, α, or β except through its third and fourth arguments. This means that P_* takes the form

$$P_*(x,y) = \frac{1}{\mu}p\left(\frac{x-a}{\mu^{1/2}},\frac{y-b}{\mu^{1/2}},\frac{\mu}{\alpha},\frac{\mu}{\beta}\right).$$

In other words, when traits are measured as a difference from their optimum in units of $\mu^{1/2}$, their distribution depends only on the ratios of α and β to μ.

We note that P_* is a probability density so we have $\int\int P_*(x,y)\,dx\,dy = 1$. Also, we can show that $\int\int (x-a)P_*(x,y)dx\,dy = \int\int \xi\tilde{P}_*(\xi,\eta)\,d\xi\,d\eta = 0$. This follows because $\tilde{M}$and $\tilde{\Phi}$ are even functions of their arguments, so from eqn (B.7) if $P_*(\xi,\eta) = \hat{P}(\xi,\eta)$ is a solution then so is $P_*(\xi,\eta) = \hat{P}(-\xi,\eta)$. Since this solution is unique we must have $P_*(\xi,\eta) = P_*(-\xi,\eta)$, which implies $\int\int \xi\tilde{P}_*(\xi,\eta)\,d\xi\,d\eta = 0$. Therefore, the mean of the first defensive trait at equilibrium is

$$\begin{aligned} x_* &= \int\int xP_*(x,y)dx\,dy \\ &= a\int\int P_*(x,y)dx\,dy + \int\int (x-a)P_*(x,y)dx\,dy \\ &= a. \end{aligned}$$

The equilibrium variance of the first defensive trait is then

$$\begin{aligned} v_* &= \int\int (x-x_*)^2 P_*(x,y)dx\,dy \\ &= \frac{1}{\mu}\int\int (x-a)^2 p\left(\frac{x-a}{\mu^{1/2}},\frac{y-b}{\mu^{1/2}},\frac{\mu}{\alpha},\frac{\mu}{\beta}\right)dx\,dy \\ &= \mu\int\int \xi^2 p\left(\xi,\eta,\frac{\mu}{\alpha},\frac{\mu}{\beta}\right)d\xi\,d\eta. \end{aligned}$$

Therefore, $\frac{v_*}{\mu}$ depends on μ, α, or β through the ratios $\frac{\mu}{\alpha}$ and $\frac{\mu}{\beta}$ only. This means that, if

μ, α, and β are increased by a common factor λ, which means that $\frac{\mu}{\alpha}$ and $\frac{\mu}{\beta}$ are unchanged, then v_* increases by the same factor λ. A similar argument can be made for the variance of the second trait.

3.7.3 Appendix C

This appendix will show that when there is no mutation force, the variances of both the first and second defences approaches to zero.

When there is no mutation, the population distribution function in the $(t+1)$-th generation is the density iteration equations (B.4):

$$N_{t+1}(x_{t+1}, y_{t+1}) = N_t(x_t, y_t)\Phi(x_t, y_t)$$

From (B.8) and (A.1),

$$N_{t+1}(x_{t+1}, y_{t+1}) = \frac{n_t\sqrt{|W_t|}}{\pi}\sum_{j=1}^{4}\gamma_j \exp\Big(-(z_t - \bar{z}_t)^T W_t(z_t - \bar{z}_t) - (z_t - \hat{a})^T F_j(z_t - \hat{a})\Big)$$

Collecting the terms for z_t,

$$N_{t+1}(x_{t+1}, y_{t+1}) = \frac{n_t\sqrt{|W_t|}}{\pi}\sum_{j=1}^{4}\gamma_j \exp\Big(-z_t^T(W_t+F_j)z_t+2(\bar{z}_t^T W_t+\hat{a}^T F_j)z_t-(\bar{z}_t^T W_t\bar{z}_t+\hat{a}^T F_j\hat{a})\Big)$$

If we complete a square for z_t, the above is equivalent to

$$N_{t+1}(x_{t+1}, y_{t+1}) = \frac{n_t\sqrt{|W_t|}}{\pi}\sum_{j=1}^{4}\gamma_j \exp\Big(-(z_t - z'_{t+1,j})^T(W_t + F_j)(z_t - z'_{t+1,j})$$

$$+(W_t\bar{z}_t + F_j\hat{a})^T(W_t + F_j)^{-1}(W_t\bar{z}_t + F_j\hat{a}) - (\bar{z}_t^T W_t\bar{z}_t + \hat{a}^T F_j\hat{a})\Big)$$

where

$$z'_{t+1,j} = (W_t + F_j)^{-1}(W_t\bar{z}_t + F_j\hat{a})$$

If we let $s_{t,j} = \exp\Big((W_t\bar{z}_t + F_j\hat{a})^T(W_t+F_j)^{-1}(W_t\bar{z}_t + F_j\hat{a}) - (\bar{z}_t^T W_t\bar{z}_t + \hat{a}^T F_j\hat{a})\Big)$, then the above is equivalent to

$$= \frac{n_t}{\pi}\sqrt{|W_t|}\sum_{j=1}^{4}\gamma_j s_{t,j}\exp\Big(-(z_t - z'_{t+1,j})^T(W_t+F_j)(z_t - z'_{t+1,j})\Big)$$

If we make it in a normal distribution form,

$$= n_t\sqrt{|W_t|}\sum_{j=1}^{4}\frac{\gamma_j s_{t,j}}{\sqrt{|W_t+F_j|}}\frac{\sqrt{|W_t+F_j|}}{\pi}\exp\Big(-(z_{t+1} - z'_{t+1,j})^T(W_t+F_j)(z_{t+1} - z'_{t+1,j})\Big)$$

which is equivalent to

$$= n_t\sqrt{|W_t|}\Theta_t\sum_{j=1}^{4}\theta_{t,j}\frac{\sqrt{|W_t+F_j|}}{\pi}\exp\Big(-(z_{t+1} - z'_{t+1,j})^T(W_t+F_j)(z_{t+1} - z'_{t+1,j})\Big)$$

$$t = 1, 2, 3, ... \tag{C.1}$$

where $\Theta_t = \sum_{j=1}^{4}\frac{\gamma_j s_{t,j}}{\sqrt{|W_t+F_j|}}$, and $\theta_{t,j} = \frac{\gamma_j s_{t,j}}{\Theta_t\sqrt{|W_t+F_j|}}$ $(j = 1, 2, 3, 4)$. Note that $\sum_{j=1}^{4}\theta_{t,j} = 1$

From the above population distribution function, the **probability density function** in the next generation can be written as

$$f(x_{t+1}, y_{t+1}) = N(x_{t+1}, y_{t+1}) / \int_{-\infty}^{+\infty}\int_{-\infty}^{+\infty} N(x_{t+1}, y_{t+1})$$

$$= \sum_{j=1}^{4}\theta_{t,j}\frac{\sqrt{|W_t+F_j|}}{\pi}\exp\Big(-(z_{t+1} - z'_{t+1,j})^T(W_t+F_j)(z_{t+1} - z'_{t+1,j})\Big)dz_{t+1} \tag{C.2}$$

$$= \sum_{j=1}^{4}\theta_{t,j}f_j(x_{t+1}, y_{t+1})$$

where

$$\theta_{t,j} = \frac{\gamma_j s_{t,j}}{\Theta_t \sqrt{|W_t + F_j|}}$$

$$\Theta_t = \sum_{j=1}^{4} \frac{\gamma_j s_{t,j}}{\sqrt{|W_t + F_j|}}$$

$$s_{t,j} = \exp\left((W_t \bar{z}_t + F_j \hat{a})^T (W_t + F_j)^{-1} (W_t \bar{z}_t + F_j \hat{a}) - (\bar{z}_t^T W_t \bar{z}_t + \hat{a}^T F_j \hat{a})\right)$$

Therefore the population distribution probability density function in the $(t+1)$-th generation is written as a combination of four normal probability density functions (each has mean $z'_{t+1,j} = (W_t + F_j)^{-1}(W_t \bar{z}_t + F_j \hat{a})$, covariance matirx $\Sigma_{t+1,j} = \frac{1}{2}(W_t + F_j)^{-1} = \frac{1}{2}(\frac{1}{2}\Sigma_t^{-1} + F_j)^{-1} = (\Sigma_t^{-1} + 2F_j)^{-1}$, $j = 1, 2, 3, 4$). Note that the integral of $f(x_{t+1}, y_{t+1})$ in respect of (x_{t+1}, y_{t+1}) equals 1, which is the property of the probability density function. Let $E_{N_j}(\cdot)$ denote the expectation of each of the four corresponding normal population distribution function. Then the **mean in the next generation** is

$$\bar{z}_{t+1} = E(z_{t+1}) = \int_{-\infty}^{+\infty} \int_{-\infty}^{+\infty} z_{t+1} f(x_{t+1}, y_{t+1}) dz_{t+1} = \sum_{j=1}^{4} \theta_{t,j} \int_{-\infty}^{+\infty} \int_{-\infty}^{+\infty} z_{t+1} f_i(x_{t+1}, y_{t+1}) dz_{t+1}$$

$$= \sum_{j=1}^{4} \theta_{t,j} E_{N_j}(z_{t+1}) = \sum_{j=1}^{4} \theta_{t,j} z'_{t+1,j} = \sum_{j=1}^{4} \theta_{t,j} (W_t + F_j)^{-1} (W_t \bar{z}_t + F_j \hat{a}) \tag{C.3}$$

The above also equals to

$$\bar{z}_{t+1} = \sum_{j=1}^{4} \theta_{t,j} (W_t + F_j)^{-1} W_t \bar{z}_t + \sum_{j=1}^{4} \theta_{t,j} (W_t + F_j)^{-1} F_j \hat{a}$$

As mentioned in Appendix A, since $\sum_{j=1}^{4} \theta_{t,j}(W_t + F_j)^{-1} W_t + \sum_{j=1}^{4} \theta_{t,j}(W_t + F_j)^{-1} F_j = I$, the mean $\bar{z}_{t+1}$ will gradually approach to the ideal phenotype as t increases.

Now for variance, we have the **covariance matrix in the next generation** is

$$\Sigma_{t+1} = E\begin{pmatrix} x_{t+1}^2 & x_{t+1}y_{t+1} \\ x_{t+1}y_{t+1} & y_{t+1}^2 \end{pmatrix} - E\begin{pmatrix} x_{t+1} \\ y_{t+1} \end{pmatrix} E\begin{pmatrix} x_{t+1} & y_{t+1} \end{pmatrix}$$

$$= \int_{-\infty}^{+\infty}\int_{-\infty}^{+\infty} \begin{pmatrix} x_{t+1}^2 & x_{t+1}y_{t+1} \\ x_{t+1}y_{t+1} & y_{t+1}^2 \end{pmatrix} \cdot \sum_{j=1}^{4} \theta_{t,j} f_i(x_{t+1}, y_{t+1}) dz_{t+1} - \bar{z}_{t+1}\bar{z}_{t+1}^T$$

$$= \sum_{j=1}^{4} \theta_{t,j} \cdot \begin{pmatrix} E_{N_j}(x_{t+1}^2) & E_{N_j}(x_{t+1}y_{t+1}) \\ E_{N_j}(x_{t+1}y_{t+1}) & E_{N_j}(y_{t+1}^2) \end{pmatrix} - \bar{z}_{t+1}\bar{z}_{t+1}^T$$

$$= \sum_{j=1}^{4} \theta_{t,j} \cdot \begin{pmatrix} Var_{N_j}(x_{t+1}) + E_{N_j}(x_{t+1})^2 & Cov_{N_j}(x_{t+1}, y_{t+1}) + E_{N_j}(x_{t+1})E_{N_j}(y_{t+1}) \\ Cov_{N_j}(x_{t+1}, y_{t+1}) + E_{N_j}(x_{t+1})E_{N_j}(y_{t+1}) & Var_{N_j}(y_{t+1}) + E_{N_j}(y_{t+1})^2 \end{pmatrix}$$

$$- \bar{z}_{t+1}\bar{z}_{t+1}^T$$

$$= \sum_{j=1}^{4} \theta_{t,j} \cdot \Sigma_{t+1,j} + \sum_{j=1}^{4} \theta_{t,j} \cdot z'_{t+1,j} z'^T_{t+1,j} - \bar{z}_{t+1}\bar{z}_{t+1}^T$$

where $\Sigma_{t+1,j}$ is the jth covariance matrix from the jth integral, $z'_{t+1,j}$ is jth mean from the jth integral. So the above is equivalent to

$$= \sum_{j=1}^{4} \theta_{t,j} \cdot (\Sigma_t^{-1} + 2F_j)^{-1} + \sum_{j=1}^{4} \theta_{t,j} \cdot z'_{t+1,j} z'^T_{t+1,j} - \bar{z}_{t+1}\bar{z}_{t+1}^T \tag{C.4}$$

Now we can use the variance iteration equations from (C.4) to get the evolution of variance across generations. As $\bar{z}_t$ approaches to the ideal phenotype $\hat{a}$ as $t \to +\infty$, so does $\bar{z}_{t+1}$ and $z'_{t+1,j} = (W_t + F_j)^{-1}(W_t\bar{z}_t + F_j\hat{a})$ $(j = 1, 2, 3, 4)$ also approaches to the ideal phenotype $\hat{a}$. Therefore the term $\sum_{j=1}^{4} \theta_{t,j} \cdot z'_{t+1,j} z'^T_{t+1,j} - \bar{z}_{t+1}\bar{z}_{t+1}^T$ in (A.11) approaches to zero, so covariance matrix (A.11) approaches to the following equation as time grows.

$$\Sigma_{t+1} = \sum_{j=1}^{4} \theta_{t,j} \cdot (\Sigma_t^{-1} + 2F_j)^{-1} \tag{C.5}$$

If there is an equilibrium, as time t grows as large as T (a very large number), the above

equation (C.5) approaches to

$$\Sigma_T = \sum_{j=1}^{4} \theta_{T,j} \cdot (\Sigma_T^{-1} + 2F_j)^{-1} \tag{C.6}$$

If we write $\Sigma_T^{-1} = \begin{pmatrix} a & c \\ c & b \end{pmatrix}$, then the equation (C.6) can be written as

$$\begin{aligned}\begin{pmatrix} a & c \\ c & b \end{pmatrix}^{-1} &= \theta_{T,1} \begin{pmatrix} a & c \\ c & b \end{pmatrix}^{-1} + \theta_{T,2} \begin{pmatrix} a+\frac{2}{\alpha} & c \\ c & b \end{pmatrix}^{-1} \\ &\quad + \theta_{T,3} \begin{pmatrix} a & c \\ c & b+\frac{2}{\beta} \end{pmatrix}^{-1} + \theta_{T,4} \begin{pmatrix} a+\frac{2}{\alpha} & c \\ c & b+\frac{2}{\beta} \end{pmatrix}^{-1}\end{aligned}$$

which is equivalent to

$$\begin{aligned}(2-\theta_{T,1})\frac{1}{ab-c^2} \begin{pmatrix} b & -c \\ -c & a \end{pmatrix} &= \theta_{T,2}\frac{1}{(a+\frac{2}{\alpha})b-c^2} \begin{pmatrix} b & -c \\ -c & a+\frac{2}{\alpha} \end{pmatrix} \\ &\quad + \theta_{T,3}\frac{1}{a(b+\frac{2}{\beta})-c^2} \begin{pmatrix} b+\frac{2}{\beta} & -c \\ -c & a \end{pmatrix} \\ &\quad + \theta_{T,4}\frac{1}{(a+\frac{2}{\alpha})(b+\frac{2}{\beta})-c^2} \begin{pmatrix} b+\frac{2}{\beta} & -c \\ -c & a+\frac{2}{\alpha} \end{pmatrix}\end{aligned} \tag{C.7}$$

Since $\alpha > 0$ and $\beta > 0$ and the determinant of the covariance matrix $ab - c^2 > 0$, so

$$(a+\frac{2}{\alpha})b - c^2 > ab - c^2 > 0, \qquad a(b+\frac{2}{\beta})-c^2 > ab - c^2 > 0, \qquad (a+\frac{2}{\alpha})(b+\frac{2}{\beta})-c^2 > ab - c^2 > 0$$

Comparing the coefficient of term $-c$ in the matrix for both side of the equation, the coefficient in the left-hand side is larger than the coefficient in the right-hand side, so we have $c = 0$, and the equation (C.7) is equivalent to

$$
\begin{aligned}
(\theta_{T,2}+\theta_{T,3}+\theta_{T,4})\frac{1}{ab}\begin{pmatrix} b & 0 \\ 0 & a \end{pmatrix} &= \theta_{T,2}\frac{1}{(a+\frac{2}{\alpha})b}\begin{pmatrix} b & 0 \\ 0 & a+\frac{2}{\alpha} \end{pmatrix} \\
&+ \theta_{T,3}\frac{1}{a(b+\frac{2}{\beta})}\begin{pmatrix} b+\frac{2}{\beta} & 0 \\ 0 & a \end{pmatrix} \\
&+ \theta_{T,4}\frac{1}{(a+\frac{2}{\alpha})(b+\frac{2}{\beta})}\begin{pmatrix} b+\frac{2}{\beta} & 0 \\ 0 & a+\frac{2}{\alpha} \end{pmatrix} \qquad \text{(C.8)}
\end{aligned}
$$

which is equivalent to

$$
\begin{aligned}
(\theta_{T,2}+\theta_{T,3}+\theta_{T,4})\begin{pmatrix} \frac{1}{a} & 0 \\ 0 & \frac{1}{b} \end{pmatrix} &= \theta_{T,2}\begin{pmatrix} \frac{1}{a+\frac{2}{\alpha}} & 0 \\ 0 & \frac{1}{b} \end{pmatrix} + \theta_{T,3}\begin{pmatrix} \frac{1}{a} & 0 \\ 0 & \frac{1}{b+\frac{2}{\beta}} \end{pmatrix} \\
&+ \theta_{T,4}\begin{pmatrix} \frac{1}{a+\frac{2}{\alpha}} & 0 \\ 0 & \frac{1}{b+\frac{2}{\beta}} \end{pmatrix} \qquad \text{(C.9)}
\end{aligned}
$$

Since $\alpha > 0$ and $\beta > 0$, the above equation cannot hold unless a and b approaches to $+\infty$ as t grows, so the matrix Σ_t grows to zero matrix as t grows. Therefore the variances of both the first and second defences approaches to zero.

Chapter 4

Coevolution of group-living and aposematism in caterpillars: warning colouration may facilitate the evolution from group-living ancestor to solitary habits.

4.1 Introduction

Animals use a rich variety of defences to protect themselves from predators. A common form of antipredator defence is protective colouration, such as camouflage or aposematic warning colouration (distinctive and often conspicuous colour patterns which advertise repellent secondary defences). For example, many dart frogs (Dendrobatidae) use bright aposematic colours to warn predators of toxic chemical defence and so avoid the costs of an attack [1]. Alternatively, many species have cryptic colour patterns which reduce their detectability, for instance, the green colouration of many arboreal snakes which blend in with surrounding foliage [5].

Living in aggregated groups can also enable many antipredator strategies, such as the 'di-

lution effect' in which individual risk of predation decreases with increasing group size, assuming that a predator selects prey in a group randomly and can't consume the whole group [13]-[15][17]. Moreover, aggregation can interact with protective colouration to influence the costs and benefits of a given strategy. For instance, when aposematic individuals gather in a group, the combined signal may be magnified and more conspicuous (signal augmentation), so the predators are less likely to attack the prey group [31]. Indeed, the evolution of aposematism has been linked to group-living for many years [2], but the directional nature of the relationship remains debated. On the one hand, according to the signal augmentation hypothesis above, aposematism should evolve first and subsequently provide selection pressure to evolve grouping to enhance the warning signal [2]-[7]. On the other hand, kin selection [8]-[11] or synergistic selection (Müllerian mimicry) [10]-[12] may be important in overcoming constraints in the initial evolution of aposematism, whereby rare (new) conspicuous individuals are eaten but their kin may survive and carry genes for aposematism. Under this scenario, grouping should evolve first (for example via the presence of local kin) and subsequently facilitate the evolution of aposematism. Hence, although a link between grouping and aposematism is established, understanding the direction of this relationship can provide insights into the underlying mechanisms.

In a well-known study of caterpillars, Tullberg and Hunter [3] attempted to answer this question using early phylogenetic comparative methods and concluded that the transition to group living is more frequent in aposematic lineages than cryptic ones. This suggests that grouping is more beneficial for aposematic than cryptic species, but their study was unable to simultaneously account for the evolutionary dynamics of both aposematism and grouping, impairing our understanding of the relationship between these traits.

The current study revisits and expands the work of Tullberg and Hunter [3], taking advantage of developments in comparative biology over the last two decades to address several limitations of that study. First, we explicitly model transitions between both colour patterns and grouping habits simultaneously using evolutionary pathway models. Second, we use the estimated transition rates from our pathway models to estimate ancestral states accounting for unequal transition rates. An inherent assumption in previous work is that the ancestral state for caterpillars was solitary and cryptic, but since the larvae of many closely related insect clades, such as Trichoptera (caddisflies), Antliophora (e.g. scorpionflies and true flies), Hymenoptera (e.g. sawflies, wasps, ants, bees) are group-living, as are some more basal lepidopteran clades, we used our ancestral state estimates to test this

assumption.

Third, Tullberg and Hunter [3] use "independent contrasts" in their analysis, however, their application of independent contracts differs from the classical independent contrasts approach described by Felsenstein [33] and neglects many branches entirely by not including them in their defined contrasts. Here by adopting Pagel's [32] approach of modelling transition rates we are able to make greater use of the information in the full tree, including branch lengths.

Fourth, phylogenetic datasets are now much more comprehensive than in the 1990s. The phylogenetic trees used by Tullberg and Hunter [3] are necessarily smaller than that used here (analysed separately for each superfamily), originate from disparate data sources (including taxonomic classification as a proxy), and do not use informative branch lengths (related to time). In contrast, here we are now able to reconstruct a single phylogeny for the whole sample using a standard set of molecular data with branch lengths related to time to provide a more powerful basis for our analyses.

Here we make use of advances in comparative biology and data availability to revisit the study in the coevolution of aposematism and grouping. Our approach enables us to more robustly test conclusions, examine potentially important assumptions made, and expand the questions asked to gain a better understanding of the system. Specifically, we aim to 1) investigate evolutionary transitions between combinations of grouping (vs solitary habits) and aposematic (vs cryptic) colouration to better understand their coevolution, 2) estimate ancestral states to infer where these transitions occurred and in what order, 3) calculate the probabilities of different states across time, the equilibrium probabilities, and the expected time that the evolutionary transition cycle around all the states, so as to predict the potential future dynamics of the system.

4.2 Methods

4.2.1 Trait data

We used data on colour pattern and grouping from Tullberg and Hunter's [3] original dataset. Colour pattern was classified as either aposematic or cryptic. Caterpillars which are strikingly marked with combinations of black and yellow, red and/or white were con-

sidered aposematic, whereas other colour patterns (such as plain green or counter-shaded) were considered to be cryptic. Grouping was classified as either group-living, where caterpillars aggregate during the whole or part of their development, or solitary if they do not aggregate. Species which lay eggs in clusters but disperse upon hatching are also treated as solitary.

The dataset we used for analyses consists of 676 species, of which 541 (80.0%) are solitary-cryptic, 82(12.1%) are solitary-aposematic, 21 (3.1%) are group-cryptic, 32 (4.7%) are group-aposematic. Note that all data used for this paper are available at https://figshare.com/s/359ff8f6c15beb68fab8.

4.2.2 Phylogenetic tree

There are five superfamilies present in our dataset: Papilionoidea, Bombycoidea, Drepanoidea, Geometroidea and Noctuoidea. Two DNA sequences, CO1 and EF-1α, were obtained for the species in our dataset from the website GenBank [26] by 1 July 2018. We were able to obtain CO1 for 667 species (98.7%), EF-1α for 227 species (33.6%), and both CO1 and EF-1α sequences for 218 species (32.2%). The accession numbers for the sequences are also available at https://figshare.com/s/359ff8f6c15beb68fab8.

For each of the five superfamilies, we aligned CO1 and EF-1α nucleotide sequences using MUSCLE [21] with default settings in the software MEGA7 [30], and concatenated the aligned sequences together using the software SequenceMatrix [20]. We use BEAST v1.10.4 [16] to estimate Bayesian trees separately for the five superfamilies. We use the generalised time-reversible model [34] as the substitution model with gamma-distributed rate variation and an estimated proportion of invariant sites [35], and we use the strict clock model [29] as the molecular clock model. The five maximum clade credibility superfamily trees were subsequently grafted together based on the higher-level topology and divergence times from the TimeTree database [22]-[25]. The divergence date for the total tree (after combining superfamily trees) was 114 million years ago and so the branch lengths were scaled to give a total tree height of this age. Note that we generate the five trees separately as a combined analysis failed to achieve convergence and (unlike our divided approach) estimated topologies which conflicted with our current understanding of lepidopteran phylogeny, for instance placing some species within superfamilies other than their own. In contrast, our 'divide and conquer' strategy estimated trees that were

generally consistent with our existing phylogenetic understanding of Lepidoptera.

4.2.3 Analysis of the coevolution of colour and grouping

We estimated evolutionary pathway models between each of the four states combining the two binary traits of aposematism and grouping following Pagel's [32] method for estimating transition rates. The transition in each state follows continuous-time Markov process, and the four states constitute eight parameters in eight pathways to estimate [32]. The dual transition in both of the binary traits is assumed to be not possible [32] (Figure 4.1), which is plausible since the simultaneous transitions in both binary states are very unlikely to happen either in the continuous-time Markov process. Pathway models were estimated via maximum likelihood using the function corDISC in the package corHMM 1.22 [19] implemented in R 3.5.1 [18].

We first estimated transition rates for a general model which has no constraints (Figure 4.1). To explicitly test for alternative evolutionary pathways we also fitted a series of restricted models in which different combinations of the transition rates were constrained to equal 0. Figure 4.1 shows one example of the restricted models in which the transition rate 'csg' (cryptic solitary →cryptic group) is set to 0 and so not possible. We have named our models such that the unconstrained model is called 'general' and constrained models are named after the rates which are constrained; for instance, we use '(csg)' for the constrained model with the transition 'csg' not possible, shown in Figure 4.1. Similarly, a model with rates 'csg' and 'gca' set to 0 is referred to as '(csg, gca)'. We have exhausted all the possible models, and overall we have $2^8 - 1 = 255$ models including the general model. We compare the evidence for each of our pathway models using Akaike's information criterion (AIC).

We estimated the ancestral state combinations using the best fitting pathway model in a maximum likelihood framework, implemented using the plotRECON function in the R package corHMM. Incorporating our results from our pathway modelling into this analysis should lead to improved estimates by accouting for any inferred constraints on the evolution of the traits.

subsectionThe state probability dynamics across time and the equilibrium The probabilities for the four binary states (solitary cryptic- sc, solitary aposematic- sa, group cryptic- gc, and group aposematic- ga) across time can be calculated using the transition rates estimated in subsection 4.2.3 (shown in Figure 4.2). Suppose $P_i(t)$ ($i \in \{sc, sa, gc, ga\}$) is

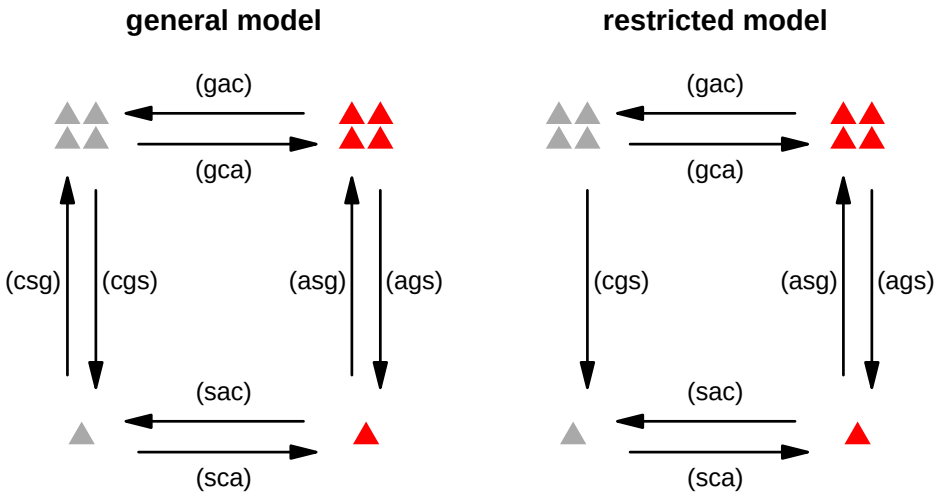

Figure 4.1: Diagrammatic representations of the general model (left) with no constrained transition rates and an example of a restricted model (csg) with some transition rates (in this case rate 'csg', cryptic solitary to cryptic group) constrained to 0. (grey, red)=(cryptic, aposematic); (one triangle, four triangles)=(solitary, group).

the probability of being in state i at time t, and $M_{i,j}$ ($i, j \in \{sc, sa, gc, ga\}$) is the transition rate from state i to state j [32], then the rate of change of probability of being in each of the four binary states across time satisfies the Kolmogorov forward equations:

$$\frac{dP_i(t)}{dt} = \sum_{j \neq i} M_{j,i} P_j(t) - \sum_{j \neq i} M_{i,j} P_i(t), \quad i, j \in \{sc, sa, gc, ga\}. \tag{C.1}$$

Equation (C.1) can also be written in a matrix form as follows:

$$\frac{dP(t)}{dt} = MP(t). \tag{C.2}$$

where,

$$M = \begin{pmatrix} -\sum_{j \neq sc} M_{sc,j} & M_{sa,sc} & M_{gc,sc} & M_{ga,sc} \\ M_{sc,sa} & -\sum_{j \neq sa} M_{sa,j} & M_{gc,sa} & M_{ga,sa} \\ M_{sc,gc} & M_{sa,gc} & -\sum_{j \neq gc} M_{gc,j} & M_{ga,gc} \\ M_{sc,ga} & M_{sa,ga} & M_{gc,ga} & -\sum_{j \neq ga} M_{ga,j} \end{pmatrix}, \tag{C.3}$$

$$P(t) = \begin{pmatrix} P_{sc}(t) \\ P_{sa}(t) \\ P_{gc}(t) \\ P_{ga}(t) \end{pmatrix}. \tag{C.4}$$

The probabilities for the four states across time can also be solved from eq. (C.2). Since the initial state at the root of the tree can be either of the four states (sc, sa, gc, ga), we will solve (C.2) separately for the four possible initial probabilities: $(1, 0, 0, 0)$, $(0, 1, 0, 0)$, $(0, 0, 1, 0)$, $(0, 0, 0, 1)$. The solution will have the form as follows,

$$\begin{pmatrix} P_{sc}(t) \\ P_{sa}(t) \\ P_{gc}(t) \\ P_{ga}(t) \end{pmatrix} = \begin{pmatrix} c_1\vec{v_1} & c_2\vec{v_2} & c_3\vec{v_3} & c_4\vec{v_4} \end{pmatrix} \begin{pmatrix} e^{\lambda_1 t} \\ e^{\lambda_2 t} \\ e^{\lambda_3 t} \\ e^{\lambda_4 t} \end{pmatrix} = C \begin{pmatrix} e^{\lambda_1 t} \\ e^{\lambda_2 t} \\ e^{\lambda_3 t} \\ e^{\lambda_4 t} \end{pmatrix}. \tag{C.5}$$

Where λ_1,λ_2,λ_3,λ_4 are the eigenvalues of M, $\vec{v_1}$, $\vec{v_2}$, $\vec{v_3}$, $\vec{v_4}$ are the right eigenvectors of M, c_1, c_2, c_3, c_4 are the coefficients solved from (C.5) given the initial probabilities at time

$t = 0$, and C is the 4×4 dimensional matrix representing $\begin{pmatrix} c_1\vec{v_1} & c_2\vec{v_2} & c_3\vec{v_3} & c_4\vec{v_4} \end{pmatrix}$.

The equilibrium probabilities can be solved using linear algebra by assuming that each of the probabilities in (C.5) does not change:

$$\frac{dP_i(t)}{dt} = 0, \quad i \in \{sc, sa, gc, ga\}, \tag{C.6}$$

with the constraint that $\sum_i P_i(t) = 1$.

4.2.4 The mean first passage time

The transition between the four binary states form a cycle (Figure 4.2). It is possible that after a certain time, the transition from one state (e.g. gc) goes around a whole cycle and back to the same state (e.g. gc) again. We can formulate a first passage time problem to calculate the mean duration of such a cycle, using the transition rates defined above. We consider the transition in the clockwise cycle, as it turns out that counter-clockwise cycles are not possible because the estimated transition rate from gc to sc is zero. If the transition travels from the state, e.g. gc, clockwise back to the same state, gc, then the whole transition is like $gc \to ga \to sa \to sc \to gc'$, where gc' represents the state gc accessed directly from sc. If T_i denotes the mean time to reach the state gc', starting from the state $(= gc, ga, sa, sc, gc')$, then considering the transitions that can take place during an infinitesimal time interval. We have,

$$T_i = dt + dt \sum_{j \neq i} M_{i,j} T_j + T_i (1 - dt \sum_{j \neq i} M_{i,j}) \tag{C.7}$$

$$\Rightarrow -1 = \sum_{j \neq i} M_{i,j} T_j - T_i \sum_{j \neq i} M_{i,j}, \quad i, j \in \{sc, sa, gc, ga\}, \tag{C.8}$$

which is to be solved under the condition $T_{gc'} = 0$. Note that the mean first passage time for a whole cycle starting from any the states (gc, ga, sa, sc) will be the same.

	K	logLik	AIC	ΔAIC	LikRatio	AkaikeWeight
(cgs)	7	-363.835	741.671	0.000	1.000	0.725
general	8	-363.835	743.671	2.000	0.368	0.267
(cgs,asg)	6	-369.900	751.800	10.129	0.006	0.005
(cgs,csg)	6	-370.794	753.587	11.916	0.003	0.002
(asg)	7	-369.900	753.800	12.129	0.002	0.002

Table 4.1: The five best models according to Akaike's Information Criteria (AIC). ΔAIC= difference in AIC between each model and the best model (AIC-AIC_{min}); LikRatio = likelihood ratio between each model and the best model (exp((AIC_{min}-AIC)/2)), sometimes called the 'evidence ratio' and gives the strength of evidence for each model as a proportion of the best model; AkaikeWeight = model probabilities (probablility of each model being the best model in the set) [27][28].

4.3 Results

4.3.1 Transition rate model comparison

The best five models chosen by the AIC method are shown in Table 1, whereby the 'best' model is that with the lowest AIC value. Model (cgs) is the best and equivalent support exists for the 'general' model since the log-likelihoods are identical and AIC values differ by exactly 2 (attributable solely to the penalty of the extra parameter which adds no more information). In keeping with the model selection statistics, these two models are actually identical since they only differ structurally by the general model having one extra parameter, but this parameter is estimated as 0 (Figure 4.2). Other models receive much weaker, in fact negligible, support. Hence we find strong support for one particular pathway model (illustrated in Figure 4.2) in which cryptic caterpillars are unable to shift from group to solitary.

4.3.2 Ancestral state estimation

The ancestral state estimation suggests that, contrary to previous assumptions, the ancestor at the root of our tree was group, although it is ambiguous whether this ancestor was cryptic or aposematic (Figure 4.3). The subsequent evolutionary history of this clade has been characterised by several transitions to the solitary and cryptic state which characterises 80% of the species in our dataset. Several of these transitions occurred in species-rich

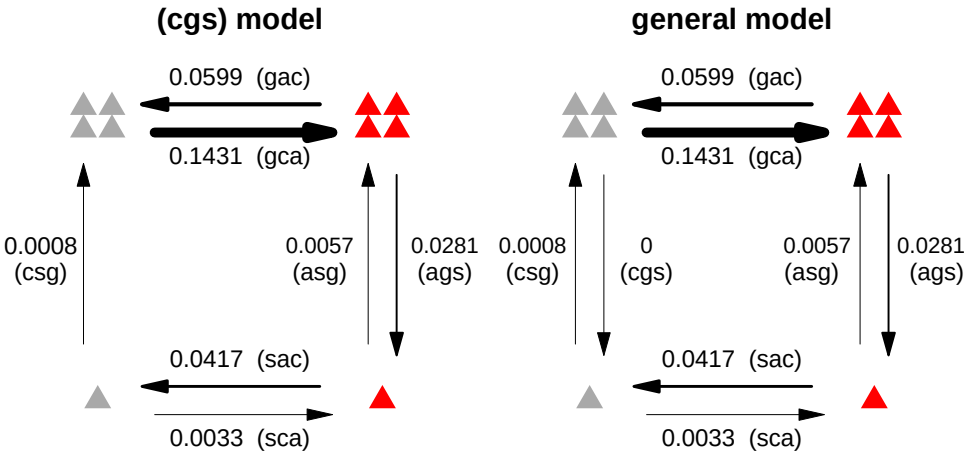

Figure 4.2: The two lowest AIC models and their estimated transition rates. The restricted model (cgs) with the pathway 'cgs' not possible is the lowest AIC model. The general model with all the possible pathways is the second lowest AIC model. Colours: (grey, red) = (cryptic, aposematic); (one triangle, four triangles)=(solitary, group). The numbers in the brackets are the indices for transition pathways corresponding to the ones in Figure 4.1.

Initial probabilities	C
$(1,0,0,0)$	$\begin{pmatrix} 0.000 & 0.042 & 0.104 & 0.854 \\ -0.001 & -0.049 & -0.034 & 0.084 \\ -0.003 & 0.003 & -0.021 & 0.021 \\ 0.003 & 0.004 & -0.048 & 0.041 \end{pmatrix}$
. $(0,1,0,0)$	$\begin{pmatrix} 0.000 & -0.761 & -0.092 & 0.854 \\ 0.002 & 0.883 & 0.030 & 0.084 \\ 0.011 & -0.051 & 0.019 & 0.021 \\ -0.013 & -0.071 & 0.043 & 0.041 \end{pmatrix}$
$(0,0,1,0)$	$\begin{pmatrix} -0.027 & 0.588 & -1.415 & 0.854 \\ 0.131 & -0.683 & 0.468 & 0.084 \\ 0.649 & 0.040 & 0.290 & 0.021 \\ -0.754 & 0.055 & 0.657 & 0.041 \end{pmatrix}$
$(0,0,0,1)$	$\begin{pmatrix} 0.012 & 0.372 & -1.238 & 0.854 \\ -0.061 & -0.432 & 0.409 & 0.084 \\ -0.300 & 0.025 & 0.254 & 0.021 \\ 0.349 & 0.035 & 0.575 & 0.041 \end{pmatrix}$

Table 4.2: Matrix C for four initial probabilities

subclades which accounts for the commonness of the strategy.

4.3.3 The state probabilities across time, the equilibrium, and the first passage time

Probabilities of different states

The equilibrium probabilities, solved from eqn. (C.6), are 85.3% for the solitary cryptic state (sc); 8.4% for the solitary aposematic state (sa), 2.2% for the group cryptic state (gc), and 4.1% for the group aposematic state (ga). The time-dependent probabilities of the four binary states across time have the form eqn. (C.5), which can be solved from eqn. (C.2). The eigenvalues λ_1, λ_2, λ_3, λ_4 are -0.209, -0.052, -0.018, 0. The matrix C in eqn. (C.5) for the four initial probabilities are shown in Table 4.2.

Eq. (C.5) together with Table 4.2 show that, over time, the probabilities approach the equilibrium probabilities (85.3%, 8.4%, 2.2%, 4.1%). The dominant eigenvalue (-0.018)

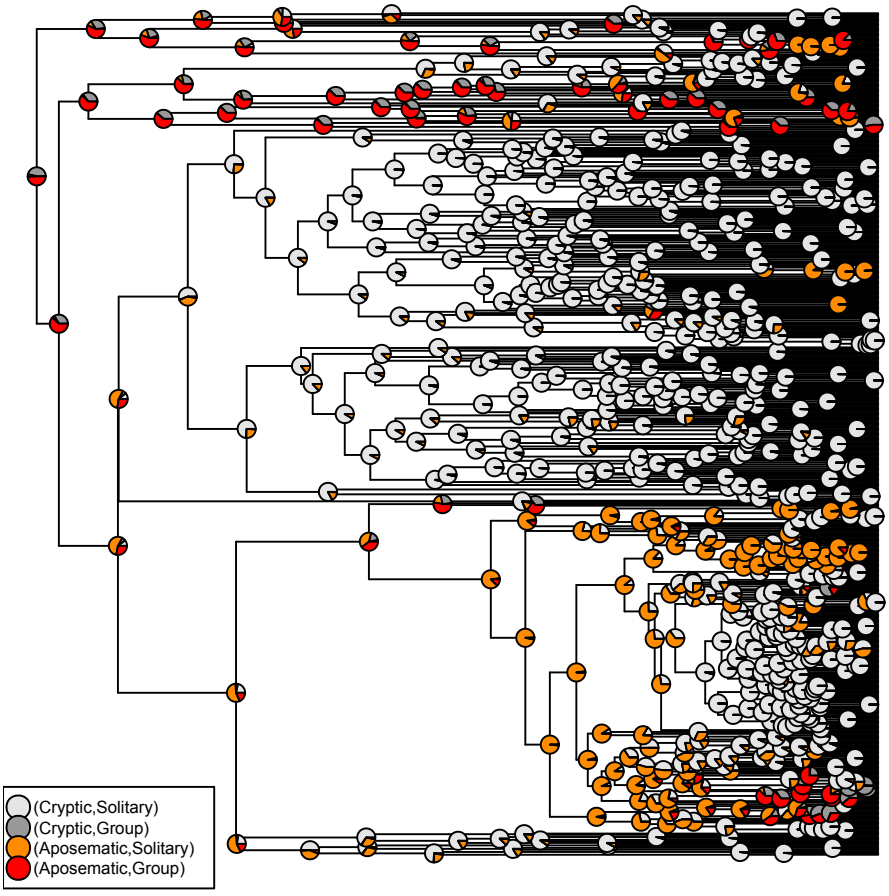

Figure 4.3: Ancestral state estimation for combinations of colour pattern and grouping. Pie charts at nodes display the relative likelihood of being in each of the four states. Note that the very common case of solitary and cryptic species arises from several transitions to this state from a group-living ancestor at the root of the tree (95% to be group-living at the root).

determines how quickly the state probabilities approach the equilibrium (since compared to -0.018, the other two eigenvalues -0.209 and -0.052 make the exponential terms more quickly go to zero as time grows). Therefore, when $t = 114$ million years, the deviations from the four state (sc, sa, gc, ga) equilibrium values are about $e^{-0.018\times114}c_3^T = 12.85\%c_3^T$ and when $t = 300$ million years, the deviations from the four state equilibrium values are about $e^{-0.018\times300}c_3^T = 0.45\%c_3^T$ (almost no deviation), where c_3 is the third column in the matrix C (e.g. $(0.104, -0.034, -0.021, -0.048)^T$ when the initial probabilities for the four states are $(1, 0, 0, 0)$). The probabilities across time given the four different initial probabilities are shown in Figure 4.4.

The first passage time

The transition rates in the clockwise direction are all higher than transition rates in the corresponding counter-clockwise direction (e.g. route gca vs. gac in Figure 4.2), and a full transition cycle can only be fulfilled in the clockwise direction, since the transition rate from grouping to solitary in cryptic caterpillars (route cgs in the counter-clockwise direction) is estimated to be zero. The mean first passage time circling clockwise, i.e. the mean time taken to evolve in a cycle from one state back to the same state, is 1465.7 million years. The mean first passage time is much longer than the time length of the phylogenetic tree we used (114 million years old) and also the time by which the state probabilities are close to the equilibrium (e.g. 300 million years, Figure 4.4). This means that the probabilities are close to the equilibrium some time before each state is expected to evolve through a full cycle. This is because the transition rate from solitary cryptic back to the group lineage (0.0008) is relatively very low, so the waiting time from solitary cryptic to group cryptic has mean of 1377 million years (which can be calculated from eq. C.8). So it is more likely to stay in the solitary cryptic state, or go counter-clockwise direction to solitary aposematic state, rather than transit to the group cryptic state.

The transient dynamics towards equilibrium

Figure 4.4 and Figure 4.2 show the dynamics of the four states. Just as can be seen in eq. (C.5), whatever the initial state is, the probabilities of the four states will approach the equilibrium.

When the initial state is either *gc* or *ga* (as estimated in Figure 4.3), the probabilities of

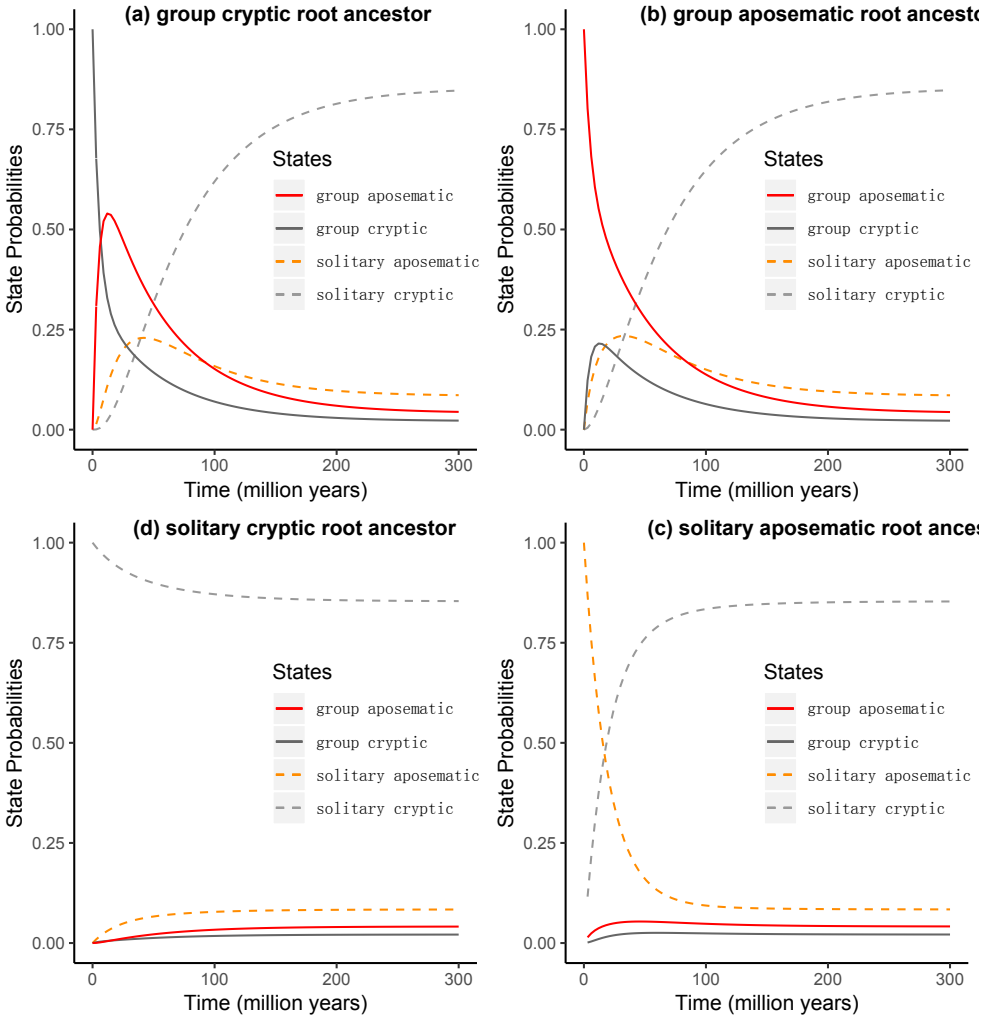

Figure 4.4: The probabilities of four binary states are approaching the equilibrium probabilities across time. Solitary-cryptic: dashed grey; solitary-aposematic: dashed orange; group-cryptic: solid grey; group-aposematic: solid red.

the other state and the state *sa* will first increase quickly from 0 to one point (the time for which can be calculated from eq.C.5), and then gradually decrease towards the equilibrium values. This is because the transition rates between *gc* and *ga* is relatively high (0.1431 and 0.0599), and there is also a moderate transition rate from *ga* to *sa* (0.0281).

Furthermore, since the transition rates away from *sc* to *gc* (0.0008) and to *sa* (0.0033) are very low, all the other states are more easily go in clockwise direction (as the clockwise transitions are all higher than the corresponding counter-clockwise transitions) towards the state *sc*, but will less easily leave the state *sc*, so the equilibrium probability for the state *sc* is the highest.

Comparing model and empirical state probabilities

The phylogenetic tree is 114 million years, so the data in our dataset are at t=114 million years. The frequency of *sc* in our dataset is 80.0%, which is lower than the equilibrium probability, and the probabilities of *sa*, *gc* and *ga* are 12.1%, 3.1%, and 4.7% respectively, which are all higher than the corresponding equilibrium probabilities. Therefore, we can predict that the probability of state *sc* is likely to increase further, and the probabilities of states *sa*, *gc* and *ga* are likely to decrease over future evolutionary time. This trend is closer to the above two graphs "gc root ancestor" and "ga root ancestor", consistent with our ancestral state estimation showing that the initial state is more likely to be state *gc* or *ga* (Figure 4.3).

4.4 Discussion

4.4.1 Group-living is likely to be the ancestral state for caterpillars

This research was initiated from the debate about whether the evolution from crypsis to aposematism is typically before or after the evolution form solitary-living to group-living. The hypothesised pathway via kin (or synergistic selection [8]-[12]) predicts that a transition from solitary to group-living comes first, and then facilitates the evolution of conspicuous warning colouration. An alternative pathway, via signal augmentation [2]-[7] predicts that the evolution of aposematism precedes the evolution of group-living since group enhances the effect of aposematism. Our results challenge the underlying premise of both of these hypotheses and instead find strong evidence for a group-living ancestral

state (leaving the evolution of solitary-living as requiring explanation). This contradicts the assumptions of previous analyses [2]-[12], and (because the ancestral colour state is so uncertain) limits our ability to answer the initial question of 'did grouping or aposematism evolve first'. Specifically, the ancestor at the root of our tree (Figure 4.3) was estimated as group living with the relative likelihood ∼0.95 (with the ancestral colour pattern is highly ambiguous). The analysis in the probability dynamics (Figure 4.4) also shows that the initial state is likely to be a group state (either group cryptic or group aposematic state) and solitary cryptic state is more stable than the other states and has the highest abundence in the equilibrium (as will be mentioned below).

4.4.2 Aposematism might facilitate the transition from group-living to solitary-living

The transition rates estimated in Figure 4.2 show that the transition is very likely to go in a clockwise direction from a grouping state to the solitary cryptic state (as the transition rates are all higher in clockwise direction compared to the counter-clockwise direction). In the two possible ancestral group states (group cryptic- gc and group aposematic -ga), it is more likely to transit from cryptic lineage (gc) to aposematic linage (ga) (rate 'gca') than the other way around (rate 'gac'). That the transition rate from gc to ga is relatively quite high supports the kin selection hypothesis [8]-[12] to some extend, since it assumes that aposematism is evolved in kin groups. This high rate probably also explains the tight relationship between the group-living and aposematism since group-living relatively rapidly leads to aposematism and hence limits the opportunity to observe cryptic group-living caterpillars, the rarest state in our dataset (3.1% of species). This is probably one of the reasons that previous research ([2]-[12]) barely connects group-living with crypsis, but rather with aposematism.

The following clockwise transition from group aposematic (ga) to solitary aposematic state (sa) (rate 'ags') is more likely to happen compared to the corresponding counter-clockwise transition from sa to ga (rate 'asg'), which may indicate costs to this strategy such as increased predation by toxin-resistant predators due to the greater conspicuousness of groups [17]. Notably, transitions from group-living to solitary-living can only happen in aposematic species (rate 'ags'), not cryptic species (rate 'cgs'). In fact, almost all transitions

between group-living and solitary caterpillars occur in aposematic lineages which suggests that aposematism may facilitate shifts in grouping vs solitary-living in both directions. Perhaps this operates by providing an additional level of protection above that conferred by group benefits, hence loosening evolutionary constraints against changes in aggregation status. Importantly, our finding that the transition rate from solitary to grouping is higher in aposematic species (rate 'asg') than in cryptic species (rate 'csg') agrees with Tullberg and Hunter's [3] finding, which is used to support signal augmentation hypothesis in their research. Our ability to recover the results of Tullberg and Hunter's original work using appropriate comparisons in our study demonstrates a congruence that adds weight to our more powerful approach and the insights provided.

The later clockwise transition rate from *sa* to *sc* (rate 'sac') is also higher than the corresponding counter-clockwise transition rate from *sc* to *sa* (rate 'sca'), which may indicate that staying cryptic is more beneficial than warning predators for solitary individuals. This perhaps because of the increased chance of being spotted and consumed (without group benefits) in conspicuous singletons, an explanation which is consistent with a kin selected (or similar) origin of aposematism since it suggests costs to being aposematic when solitary.

The net result of all these transition rates are that colour pattern (horizontal see transitions in Figure 4.2) is far more evolutionarily labile than aggregation propensity grouping pattern (see vertical transitions in Figure 4.2), and the transitions are more likely to go from the possible ancestral group state to the later relatively stable solitary cryptic state in clockwise direction, and so the loss of group-living trait might be facilitated by the protection of warning colours. Furthermore, the transition rates between group states (group/solitary) is higher in the aposematic species than in the cryptic species, and the transition rate between two colour states (aposematism/crypsis) is higher in group states than in solitary states. This is probably driven by the synergistic effects of aposematism and group-living in terms of increasing conspicuousness, and vice versa for crypsis and solitary-living. This agrees with Tullberg, Leimar and Gamberale-Stille [4] who found no difference in attack rates on cryptic and aposematic prey in groups, but the attack rate on the aposematic prey is significantly lower than on the cryptic prey in solitary individuals. This also agrees with Alatalo and Mappes [10] who showed that the relative mortality caused by predators was more similar between group aposematic and group cryptic unpalatable prey than between solitary aposematic and solitary unpalatable prey.

4.4.3 Solitary cryptic caterpillar will be the most abundant at the equilibrium

The equilibrium probabilities for the four states – *sc*, *sa*, *gc*, *ga* – are 85.3%, 8.4%, 2.2%, 4.1%, respectively, and over time, the probabilities of the four states will go towards the equilibrium values no matter what the ancestral state is. As mentioned above, the solitary cryptic species is unlikely to be the ancestral state, but this state will become more common and then gradually approach the equilibrium abundance (85.3%) since transitions are more likely to go from the group state towards and then stay in the solitary cryptic state. The frequencies of the other states will grow at first, since the transition rates between the three conspicuous states (*gc*, *ga*, *sa*) are relatively high, and then after certain points, the probabilities of all the states will fall towards their corresponding equilibrium values.

The transition of a full clockwise cycle is expected to last 1465.7 million years, which is much longer than the time of the tree (114 million years), and the time that the state probabilities take to approach equilibrium (e.g. 300 million years in Figure 4.4). This means that the state probabilities will be close to equilibrium a long time before the evolutionary transitions have run a full clockwise cycle. This is because the transition rate from *sc* to *gc* is very low (rate 3), so it is more likely to stay at the state *sc* for long or even transit counter-clockwise to the *sa* state rather than finish the full clockwise cycle back to the group states. Since both the transition rates away from *sc* (rate 3 and 5) are lower than the other positive rates, it will typically stay longest in the state *sc* compared to the other states.

4.4.4 Implications, limitations and future work

The pattern of highly observed frequencies of solitary cryptic states (combined with less informative comparative methods) may be why previous research focused on understanding the evolution of aposematic group-living animals from a solitary cryptic ancestor ([2]-[12]). Hence, future work to understand the loss of group-living and so the evolution of solitary life may prove fruitful. Since we find solitary-living only originates in aposematic species, not directly from cryptic lineages, we specifically encourage future work to understand how aposematism might facilitate the loss of group-living. We suggest one possibility is that being solitary is relatively risky and so reducing predation risk with warning signals facil-

itates the loss of group-living by compensating the added risk with another defence which deters attacks. Alternatively group-living may not increase conspicuousness by as great a magnitude in cryptic species than aposematic species, such that selection for switching grouping strategies is lower in cryptic lineages. Under this scenario, other benefits of group-living may prevent its loss in cryptic species, whereas the balance of costs and benefits of group-living in aposematic species may be more similar to those of solitary life. In any case, our results provide new insights into the coevolution of protective colouration and grouping tendencies in a long-standing model system and in doing so show the benefit of revisiting classic studies in ecology and evolution using newer and more powerful methodological approaches.

Caterpillars have many advantages for studies such as ours, hence they established as model systems for antipredator mechanisms. However, Macrolepidoptera is a very large clade containing over 90,000 described species, so one limit in our research is that our analysis is based on the dataset of Tullberg and Hunter [3] and we assumed that the dataset is reasonably unbiased in sampling species with respect to their traits, and sufficiently informative to draw general conclusions about the coevolutionary dynamics of the group and colour states. However, it is possible that the dataset is strongly biased and the results that we find does not tell the real underlying patterns. Nevertheless, the datasets in some other studies [36, 37, 38, 39] have also shown the similar pattern of colour and gregarious states of caterpillar as Tullberg and Hunter's [3]. Moreover, Tullberg and Hunter's dataset [3] contains five different superfamilies, and we think that data are unlikely to be biased in a consistent way across the five superfamilies can be less likely given their different lifestyles and general ecology.

4.5 Conclusion

This research revisits the classical debate about the evolutionary order between aposematism and group-living. Our results challenge the earlier assumption that aposematism and group-living are derived states from ancestral cryptic and solitary-living caterpillars. Based on analyses of transition rates of colour and grouping states, we proposed that aposematism might act as a facilitator to the solitary habits from grouping habits, perhaps by offsetting the risk incurred by losing the protective benefits of grouping. Solitary crypsis is the most stable state and has the highest abundance in the equilibrium compared to

the other states. Our results also provide new avenues for future research focused on how aposematism colours and perhaps other secondary defences, might facilitate the evolution from group-living to solitary-living in animals.

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Chapter 5

Comparing the diversification rates of defence and non-defence species

5.1 Introduction

Speciation (the formation of new species) might happen for many different reasons. It might happen when an interbreeding population is separated by some ecological barriers into several populations. Here, subpopulations may not have enough chances to interbreed, so gradually evolve to become two different species [1]. It might also start even if there is no distinct ecological barrier, but a population is living in a large area, so the individuals living in one part of the area can have less chance to interbreed with individuals living in another part of the area. The gene flow between these two groups will be less, which gradually causes reproduction isolation [2]. It could also happen when the inhabited ecological environment is different for certain groups in the population, and they experience different selection pressure, so evolve to become different species [1]. Furthermore, the new evolution of new traits in certain groups might change the selection pressure to them, and so they might evolve to become unable to interbreed with the rest of the population [3, 4].

On the other hand, extinction (the termination of existing species) might be caused by the change of environment (e.g. climate change, loss of food, pollution) [5], loss of habitat [6], natural enemies (e.g. predators, parasites, pathogens), competition from other species

(e.g. species with better traits, invasive species) [7], loss of food [8], and so on.

The terms "speciation rate" and "extinction rate" are usually used by biologists to describe the increasing or the decreasing rates of change in a number of species [9], and the term "diversification rate" is used to measure the net rate of change (i.e. the net difference between speciation and extinction rates) [10, 11]. It is important but also can be challenging, to determine what influences diversification rates. Like the causes of speciation and extinction, researchers often focus on the patterns of diversification variation with the ecological niches and characteristics of organisms. Broader ecological niches are considered to be associated with larger populations, which have higher chances to speciate into new species [12], and lower chances of becoming extinct than smaller populations [13]. In addition, broader ecological environments can facilitate allopatric speciation (which happens when the gene flow is blocked by certain barriers, such as rivers or mountain ranges) [1]. Related factors with expended niches, such as a larger geographical range size [2], or disparate ecological environments [1], are found to be influential. Characters such as self-incompatibility –which is related to increased genetic diversity [14, 15]–or floral asymmetry, which is associated with the chance of character displacement, are factors that increase diversification rates [16]. Other traits, such as defences [17], biotic dispersal growth [18], pollination systems, and life forms (e.g. herbs, shrubs or trees)[19] are also found to be influential.

Defence is one of the traits that is found to be associated with variations in diversification rates. The idea comes from Ehrlich and Raven [17], who predicted that a new defence can help the organisms enter a new adaptive zone, in which they are protected from predators' attacks, so evolutionary radiation might follow ("escape and radiate" [20]). The population with new defences can grow not only because the defence traits protect the population in the same ecological area, which might be followed by sympatric speciation [3, 4], but also because the population with the defence traits can expend their ecological habitats [21, 22], which might lead to allopatric speciation [1]. At the same time, due to the protection by defences, the extinction risk could be lower. Therefore, the net diversification rates (the difference between speciation and extinction rates) increase.

Ehrlich and Raven [17] have been cited many times (more than 4490 times) by other researchers. However, the evidence for their "escape and radiate" theory is comparatively small. One way to test the theory is, as was proposed in [17], to see whether there is a

significant increase in the diversification rate of the clade where a new defence evolves (compared to the sister-clade without the corresponding new defence)in the ancestral estimated phylogenetic tree (sister-clade analysis). For example, increases in the diversification rates are found in the insect lineages that have new chemical defenced host plants [23, 24, 25]; on the other hand, plant lineages that have new mutualisms with chemical defenced insects are also found to have higher diversification rates [10, 20]. Compared to chemical defences in mutualised plants and insects, the evidence about chemical defences in non-mutualised organisms is less consistent. For example, in the study of plant diversification in Farrell [3], it was found, that the lineages with the defence resin canal have higher diversification rates than the lineages without the defence, but Vamosi [26] found no significant relation between resin canal and diversification rates. Arbuckle and Speed [11] found that both the speciation rates and extinction rates increase in the amphibian lineages with chemical defences, but that the net diversification rates decrease in the lineages with chemical defences. Agrawal et al. [27] also found a negative relation between the investment in milkweed chemical defence and diversification rates.

Another way to test the theory is not just to focus on the sister-clades, but to calculate both the speciation rates and extinction rates for traits in the full phylogenetic tree (Binary State Speciation and Extinction –BiSSE [28]). Using this method, the relation between diversification rates and chemical defences is similar to using the above methods. Peña and Espeland [29] found higher diversification rates in butterflies which feed on toxic plants compared to those which do not. Increased diversification rates are also found in plants mutualised with chemical defenced insects [10, 20]. Armbruster et al. [30] however, found no evidence between diversification and chemical defences in vines.

Compared to the chemical defences, the association between aposematic defences and faster diversification rates is more consistent, although the evidence is also scarce. Unlike aposematic species, cryptic species may be constrained behaviourally. For example, they may have narrow foraging niches because they can match relatively few backgrounds, or they may be constrained to feed nocturnally. Aposematic species may lose these constraints and be able to use expended and diverse ecological opportunities. Przeczek et al. [31] compared 14 sister clade pairs of amphibians, spiders, and insects and found evidence for increased diversification in aposematic clades. Arbuckle and Speed [11] used BiSSE and found that diversification rates are higher in the amphibians with conspicuous colours. Also, increased acoustic diversification is found in poison frogs with aposematic defences

using BiSSE (Santos et al., [32]).

Living in groups can benefit organisms with regard to their foraging, survival, or defence behaviours [33]. The reason why group-living behaviour can persist could be that individuals can have higher fitness by living in group since their genes can be passed on to the next generation not only from the individuals themselves but also from their nearby relatives, so they might choose to be altruistic to enhance the genetic fitness of both the recipient of the act and the altruists themselves ("inclusive fitness"). [33]. For caterpillars, the group-living behaviour can be associated with cooperative living and foraging (e.g. *Malacosoma americanum*) [34], and can also function as a defence. Since caterpillars are soft and vulnerable, and they move slowly relative to many other animals, a group can help to protect them from their natural enemies [35]. The yellowneck caterpillar (*Datana ministra*)makes a U-shaped posture to make ovipositor more difficult for some parasitoids (braconid wasps, or tachinid flies) [36]. This defence behaviour can be simultaneously displayed by other caterpillars in the group, even if they are not attacked by parasitoids. The sharing of danger signals can not only inform the nearby caterpillars that are not attacked to start to defend themselves but also the group can form a large menacing defence together, which can frighten parasitoids or even predators away [36]. Besides group displays, caterpillars can also stay together to form a large defence (e.g. a shelter, or a pattern) for their protection [35]. Also, group-living is also found to enhance the effect of some defences, such as aposematic defences [37, 38, 39, 40, 41, 42] or chemical defences [43]. In addition, the individuals' fitness can also be affected by the risk-dilution effect in the group (because per capita risk decreases with group size). For example, although a larger group size can increase the detection risk from parasites in leaf miner caterpillars (*Antispila nysaefoliella*), it also decreases the post-detection risk while individuals are hiding in groups [44, 45, 46]. Therefore, like the other defences mentioned above, group-living defences might also be associated with diversification rates, although we do not find any literature that tests this.

In this chapter, the BiSSE method is used to test whether the two defences, aposematism and group-living, can influence the diversification rates, to provide further evidence for Ehrlich and Raven's "escape and radiate" theory [17, 20]. We will show whether aposematism can increase diversification rates, as suggested by previous research [11, 31, 32], and whether group-living can increase diversification rates, which is a new evidence area for the "escape and radiate" theory. We will however not pool one defence trait while analysing

the other one as the dataset we used is not large enough to do this.

5.2 Method

5.2.1 The tree and the traits

The phylogenetic tree and the traits are the same as those in the previous chapter. We used the genes CO1 and EF-1α from Genbank to make the Bayesian phylogenetic tree and used the trait dataset from the research by Tullberg and Hunter [38].

5.2.2 Net diversification rate

We use the method proposed by Maddison, Midford and Otto [9], which introduces the "BiSSE" model (Binary State Speciation and extinction). In our case, we use the model to solve the speciation and extinction rates for the state "aposematism" and "crypsis" and the state "group-living" and "solitary-living" respectively of the Macrolepidoptera order. The net diversification rate is the difference between speciation rate and extinction rate. The functions "make.bisse" and "find.mle" in [47, 48, 49] in the package "diversitree" [50, 51] in R 3.5.1 [52] are used to obtain the maximum likelihood (ML) values for all of the rates.

For the "BiSSE" analysis, we also need to know the smallest single clade which contains all of the species we used; in particular, the fraction of this clade that covers our dataset. The smallest clade which covers all of our species has 92,100 species, with the five superfamilies, Papilionoidea, Bombycoidea, Drepanoidea, Geometroidea, Noctuoidea each having approximately 14,000, 3,400, 700, 22,000, 52,000 species, respectively [53]. Therefore the proportion of our sample to this full single clade is 0.7% (=676/92,100).

Since the total numbers of the five superfamilies are only known approximately, as the Lepidoptera Order is still unsolved, we are unsure of the exact proportion value of our dataset. We, therefore, obtained the results for a range of different assumed proportion values above and below 0.7%, to see if the different proportion values make any difference to the result. We conducted the analysis for the proportion range from 0.1% to 1.5% (0.1, 0.3, 0.5, 0.7, 0.9, 1.1, 1.3, 1.5, specifically). Then we compared whether the diversification rates for the aposematism state were consistently higher than the diversification rates for

the crypsis state; and whether the diversification for the group-living state was consistently higher than the diversification rates for the solitary-living state for this proportion range 0.1%-1.5%.

When we obtain the ML values for the rates for different proportion values between 0.1% and 1.5%, we use these values as the initial values and conduct the mcmc analysis [54] for those proportion values to obtain the diversification rate posterior density distributions for the aposematism/crypsis states and group/solitary-living states. Then, we use the posterior distributions to see whether the two rates are different.

5.3 Results

5.3.1 Aposematism and crypsis

Diversification rates of aposematism and crypsis for different assumed proportion values

Both the speciation rates and extinction rates with the defence aposematism are consistently higher than without the defence aposematism for all of the assumed proportion values from 0.1% to 1.5% (Figure 1 left). Equivalently, the net diversification rates with the defence aposematism are consistently higher than without the defence aposematism for all of the assumed proportion values from 0.1% to 1.5% (Figure 1 right).

The speciation and extinction rates are higher when the proportion values of the species that are covered in the samples are lower since, in this case, the actual number of species in the real world is higher, so the corresponding speciation rates and extinction rates will be higher. The net diversification rates are relatively stable and do not change much with the proportion values.

MCMC posterior density distribution of diversification rates of aposematism and crypsis for different assumed proportion values

The mcmc posterior density distributions show that the diversification rates with aposematism are distributed much higher and with larger variance than the diversification rates

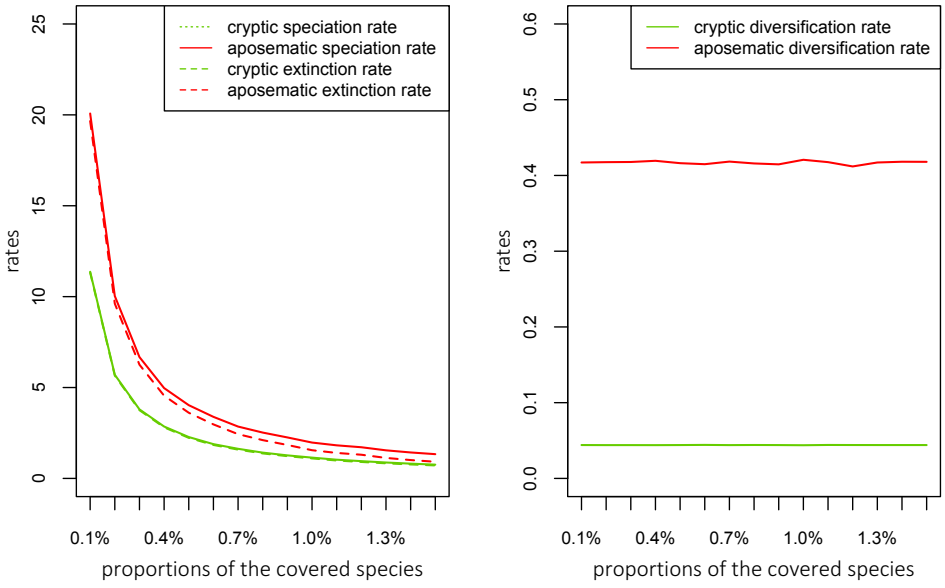

Figure 5.1: The speciation and extinction rates (left), and net diversification rates (right) of cryptic and aposematic lineages with different values for the assumed proportions of species that are covered in the samples.

without aposematism, with a smaller variance for all of the proportion values (Figure 2).

5.3.2 Group-living and solitary-living

Diversification rates of group-living and solitary-living for different assumed proportion values

Like the results above for aposematism and crypsis, both the speciation rates and extinction rates with the defence group-living are consistently higher than without the defence group-living for all of the proportion rates from 0.1% to 1.5% (Figure 3 left). The net diversification rates with the defence group-living are consistently higher than without the defence group-living for all the proportion rates from 0.1% to 1.5% (Figure 3 right).

The speciation and extinction rates are higher when the proportion values of the species that are covered in the samples are lower. The net diversification rates are relatively stable.

MCMC posterior density distributions of diversification rates of group-living and solitary-living for different assumed proportion values

The mcmc posterior density distributions show that the diversification rates with group-living are distributed much higher and with a larger variance than the diversification rates without group-living with a smaller variance for different proportion rates (Figure 4).

5.4 Discussion

Diversification rates with and without the defence aposematism and the defence group-living are compared in this research. Diversification rates with the defence aposematism /group-living are consistently higher than the diversification rates without the defence aposematism/group-living. This agrees with Ehrlich and Raven's"escape and radiate" theory [17], that defences can be associated with higher diversification rates. Specifically, the results about the aposematism agree with the previous research in its positive association with the diversification [11, 31, 32]. The association between diversification rates and group-living is to my knowledge a new finding, and here we find that group-living also helps to increase the diversification rates.

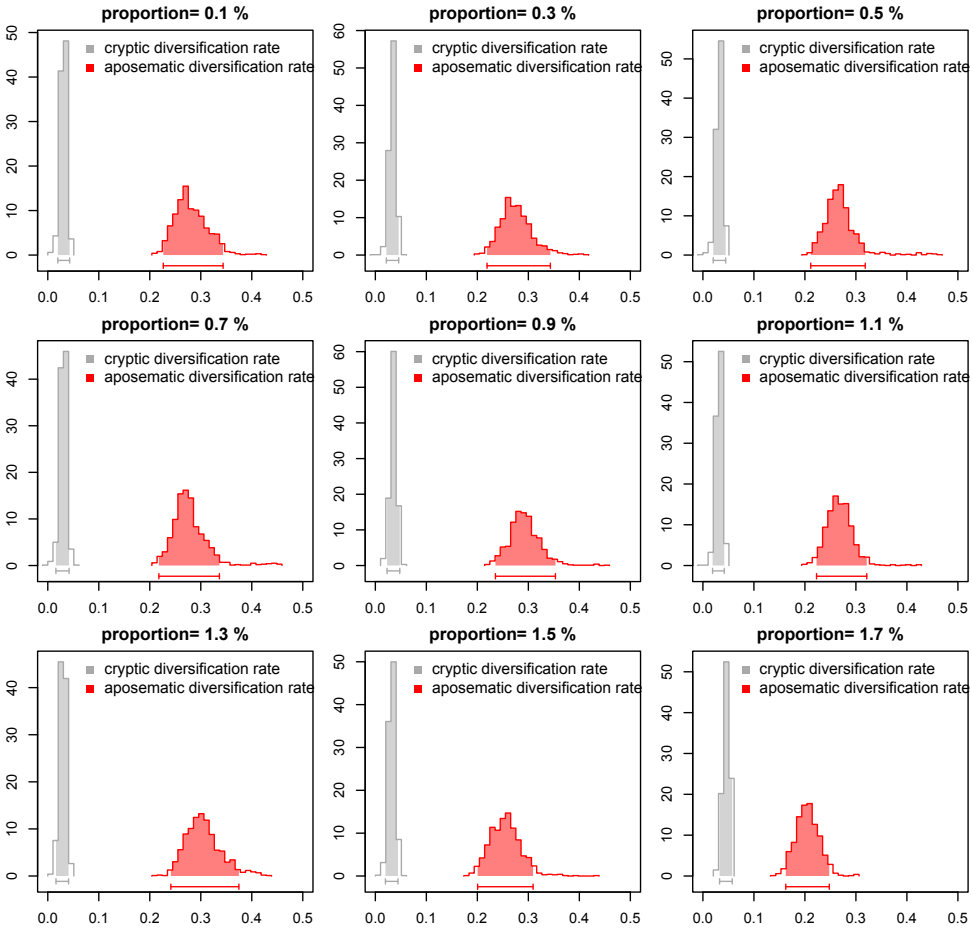

Figure 5.2: The posterior probability density functions for the diversification rates of both cryptic and aposematic lineages with different values for the assumed proportions of species that are covered in the samples.

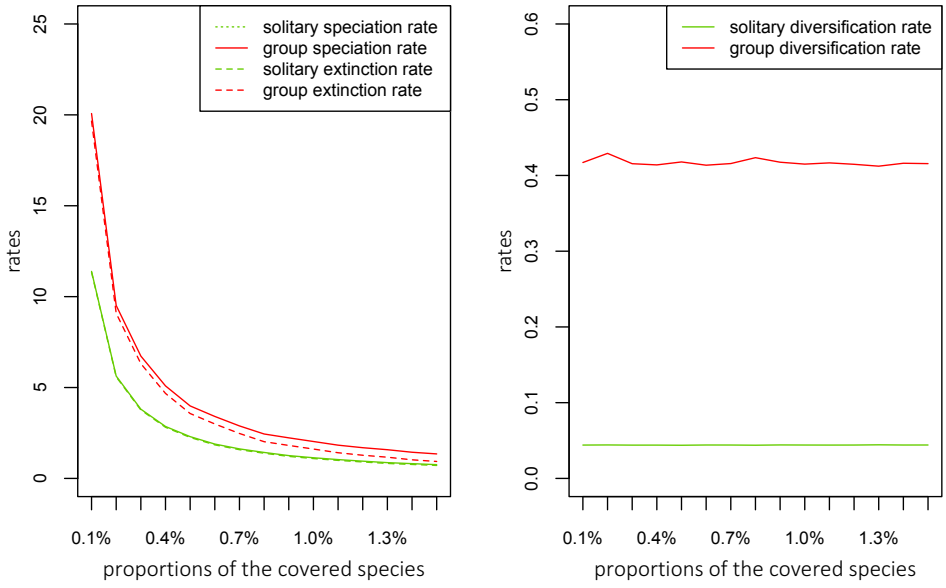

Figure 5.3: The speciation and extinction rates (left), and diversification rates (right) of solitary-living and group-living lineages with different values for the assumed proportions of species that are covered in the samples.

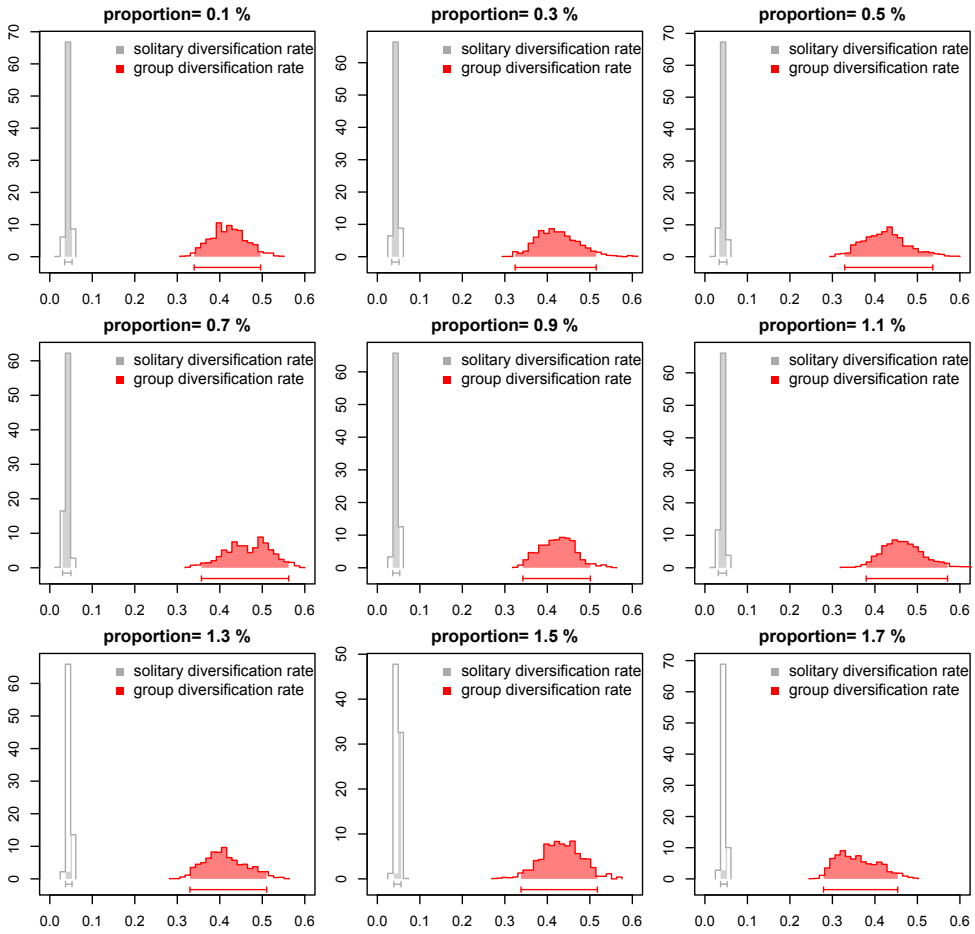

Figure 5.4: The posterior probability density functions for the diversification rates of both solitary-living and group-living lineages with different values for the assumed proportions of species that are covered in the samples.

The association between group-living and diversification rates has been barely studied before. We hypothesised that group-living can function as a defence, in the sense that it can dilute the risks each individual faces and therefore increase individuals' survival rates [44, 45, 46], and also a group itself can function as a huge defence [36, 35]. The findings here about the association between group-living and diversification rates also agree with Ehrlich and Raven's "escape and radiate" theory, as we expected [17]. However, since group-living has many functions other than defences, it is possible that it is the other roles of group-living that influence diversification rates more than its defence role. For example, some caterpillars might gather together mainly for cooperative living and foraging [34] and, compared to defending against enemies, diseases are more likely to be transmitted in their groups which brings more risks to the group [55, 56]. The overall effect of these roles of group-living might still help to increase the species' survival and reproduction rates, and therefore the population size grows, which might be followed by higher diversification rates. So here we have a positive relation between group-living and diversification rates but, since group-living has many other functions, we are unsure whether it is its defence function that helps to increase diversification rates.

Compared to the consistent findings of the association between aposematic defences and diversification rates in this and previous research, the findings of the relation between chemical defences and diversification rates, however, were ambiguous in the previous research. The reason for this might be that the chemical defences are variable, and therefore their relation with diversification rates can be complicated. Chemical defences, as secondary defences, can be more variable compared to first defences(e.g. physical, morphological defences, etc), either in their quantities, and their components [57, 58, 59, 60]; therefore, treating chemical defences using binary traits (chemical defences vs no chemical defences)might have simplified their influence on diversification rates; whereas the first defences can be less variable [59], so treating them as binary traits (defences vs no defences) is relatively plausible. Since the current evidence is limited, we are unsure whether the "escape and radiate" theory [17] is true for some defences, or if it is a questionable theory. It will be helpful for future researchers to offer more evidence in this area.

The species' mutualistic defence relationship with another species might further help to explain the "escape and radiate" theory [17]. As mentioned in the introduction to this chapter, the chemical defences obtained from other mutualised species are found to have a consistent association with faster diversification rates—those insects with chemical defences

gained from the host plants have higher diversification rates than those that lack these [23, 24, 25]. At the same time, the plants that have insects in a mutualistic defence relationship are also found to have faster diversification rates [10]. It is possible that the defences that function more effectively might have a closer relationship with diversification rates (e.g.the defences that mutualise with the traits of other species;or first defences, such as aposematism, that are predicted to be more effective since they are more often used than the secondary defences [59, 60, 61, 62]).

One of the limit of this study has been mentioned in subsection 4.4.4. We have assumed the dataset from Tullberg and Hunter [38] is reasonably unbiased with respect to their traits, however, certain bias of the dataset compared to the large Macrolepidoptera clade might be unavoidable. Nevertheless, we find that studies such as [35, 63, 64, 65] have shown the similar trait patterns as Tullberg and Hunter [38], also Tullberg and Hunter [38] includes five different superfamilies which can be less likely to be biased in a consistent way given their different lifestyles and general ecology. Another limit is that the dataset covers about 0.7% of the species of the species in the smallest clade that contains all of the species in our dataset, which is a small proportion value and and might influence the results associated with diversification rates. However, we have tested a wide range of proportions from 0.1% to 1.5% which covers 0.7%, which have shown consistent results for both aposematism (which is also consistent with other studies [11, 31, 32]) and group-living in two separate studies here (Figure 5.1, 5.2, 5.3, 5.4). Therefore the data has certain consistence in explaining these corresponding defence traits associated with diversification.

If one defence such as aposematism or group-living is able to increase diversification rates, we can expect that species with both defences will be more likely to increase their diversification rates than those with only one defence. In this case, it would be helpful to test whether species with two defences (both aposematism and group-living) have higher diversification rates than species with one defence (either aposematism or group-living). However, in our research, the species with aposematic defences account for $(82+31)/676 = 16.7\%$ and the species with group-living defences account for $(21+32)/676 = 7.8\%$ of the total dataset used, which means that the sample size will be much smaller than what was used above (0.7%). We suspect the new sample sizes will be relatively small for this research. Therefore, the same research could be better carried out with species whose phylogenetic trees are smaller and more fully solved than the Macrolepidoptera Order (e.g. fish families).

Ehrlich and Raven's "escape and radiate" hypothesis [17], that defences are associated with faster diversification rates, is logical and has consistent evidence for some defences, such as aposematism. However, the evidence for some other defences is less consistent, such as chemical defences. Which factors can influence diversification rates is quite a complicated question. Not only defences, but other factors such as other traits and niches might also influence diversification rates. It can be difficult when these factors are interacting with each other so the effect of defences on diversification rates might be less obvious. It is especially the case when the defences are less effective for the species in their defending functions. For example, chemical defences, as secondary defences, have fewer chances of being used compared with other earlier defences since they are deployed later, so they appear to be more variable and the selection force on them is lower [59]. Therefore, their effect on the diversification rate could be less than the other earlier defences, such as aposematic defences. Also, how to deal with the effect of variable defences on diversification rates is another question to consider, which could be more complicated than dealing with the effect of fewer variable defences.

5.5 Conclusion

Here we have revisited Ehrlich & Raven's [17] "escape and radiate" hypothesis regarding the association between diversification rates and two defence traits aposematism and group-living. Our results agree with their hypothesis that both of these traits are associated with faster diversification rates. The results about aposematism is consistent with several previous studies [11, 31, 32], and the results about group-living is new to our knowledge. Further studies are suggested in using certain smaller and more fully solved clades to test the same hypothesis and can also test whether the diversification rates is even faster with multiple defence traits than one single defence trait.

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Chapter 6

Discussion

6.1 Conclusions and Implications

The current literature about multiple defences usually focuses on cases in which each defence is-deployed individually in different circumstances (e.g. towards different enemies [1, 2], in disparate environments [3, 4, 5], or during different periods of individuals' lifetime [6, 7]). However, the connections between defences are much less studied. Defences can work synergistically with each other, so the function of at least one defence is enhanced by other defences [8, 9, 10, 11, 12]. It is found that the synergistic effect of multiple defences can help victims to survive better in the victim-exploit competition [13]. Besides synergistically operating defences, multiple defences are also connected when they are deployed sequentially since they act one after another. Predators can launch a sequence of attacks against victims; e.g. detection, identification, approach, subjugation, and consumption [14]. Victims could have better a chance of survival when they can respond effectively and correspondingly towards each level of attack [15]. This thesis focus on the connections between defences. Chapters 2 and 3 study the sequentially-deployed multiple defences, while Chapters 4 and 5 study the synergistically working multiple defences. In this thesis, the evolutionary reason, the distribution variances, the coevolution between defences, and the association between defences and diversification were explored. Here I reflect on each.

6.1.1 Sequential defences

Chapter 2 studies the evolutionary reason for multiple defences and the trade-off between defences in the sequential deployment scenario. Some organisms use only one defence very often, whereas other organisms tend to apply a sequence of several defences. The question is why do organisms invest in multiple defences rather than in one single "super-defence"? One reason for this might be that sequential multiple defences could be better than one "super-defence" in protecting victims in response to sequential enemy attacks. Another reason could lie in the effect of the sequence deployment of defences; when the earlier defences are breached, the victims can still escape the enemies when the later defences hold.

In Chapter 2, I used a mathematical model to explore the evolutionary reason for the sequential deployment of defences. The mathematical model is used to find the optimal investment allocation strategy in each defence. I find that whether the optimal strategy is to invest in multiple defences, or to invest in only one single defence is dependent on the investment function that converts victims' fitness into defences. So it can be expected that the reason why some organisms tend to use only one defence, but other organisms tend to use multiple defences, might be that the investment functions between defences are different.

I also find the trade-off between the investment in defences. The investment in early defences is typically more than the investment in later ones. This happens because the earlier defences have a higher chance of being used than later defences since they are deployed earlier. This result agrees with Endler [14], who also argues that prey should generally invest preferentially in earlier deployed defences than later ones.

The model improves the model in Broom [16] as a general form of investment function rather than a specific linear investment function is used, which might not be exactly what the victims perform. I generalise the number of defences to n rather than only two, as used in Broom [16], since some organisms in the real world have more than two levels of sequential defences. So the findings here can be applied to understand the defence investment allocation strategies in a wider range of organisms.

Chapter 3 focuses on the defence phenotype distributions in sequential defences. Defences are observed as having variable phenotypic appearances [17, 18]. I used a mathematical

model to explore the evolution of variance across time under the forces of both selection and mutation. Both mathematical analyses and numerical simulations are used to find the equilibrium variances in the mutation-selection balance in earlier and later defences. The results show that the position of the defence in the sequence influences the variance, and typically the earlier defence evolves to have less variance than the later defence. These results can help to explain why the secondary defences (e.g. chemical defences) are found to be very variable both in quantity of toxin and in the chemical constituents [17] whereas many earlier-acting physical defences such as aposematic colours, thick epidermises, and thorns appear almost identical in the same organisms. In addition, the defence effectiveness for protection can influence the equilibrium variance in such a way that a more effective defence decreases the equilibrium variance, and higher tolerance of deviation from the ideal phenotype in defences can increase the equilibrium variances.

These results agree with the results in the empirical meta-analysis [19] and field-work [20] studies. Both found the correlation between variations in later defences (e.g. chemical defences) and variations in plants' damage from herbivores are not significant, but variations in earlier defences (e.g. plant or leaf size, trichomes) are significantly related with the variation in plants' damage. This might be because the deployed position of earlier and later defences plays an important role in protection as Endler [14] proposed and also as our model suggests, so the earlier defences have higher chances so higher influence on fitness values. This might also be because the effectiveness is higher and the tolerance of deviation from the ideal is lower in earlier defence than in later defence, so the effect of resistance in the earlier defences is better than that in the later defences also indicated in our model.

6.1.2 Synergistically-acting defences

Sequential defences can be connected by acting one after another. Synergistically-acting defences have another way of connection since defences can enhance the effect of each other. For example, chemical defences and aposematic defences can enhance the effect of each other, since aposematic colours constitute further visual cues of unseen chemical defences, so their communal effect is better than the effect of each one of them individually [8, 9, 10, 11, 12].

Since the evolution of new traits is usually instantaneous compared to the persistence of existing traits, the simultaneous evolution of two traits can be rare. It is therefore worth considering which of the two synergistic defences evolved earlier than the other. For the above example, chemical defences in caterpillars might have evolved before aposematism because chemical defences themselves can protect victims, but aposematic defences themselves might attract the attention of predators, which then results in predator attack.

For the two traits considered (aposematism and group-living), previous research has different predictions about the evolutionary order of the two traits. The kin selection hypothesis suggests that the evolution of aposematism happens before group-living since aposematism is likely to evolve in kin groups. The signal enhancement hypothesis suggests that since group-living enhances the effect of aposematism, the evolution of group-living evolved after aposematism. Even so, I suspect that there could be dynamic coevolution between the two. Since the larvae of many closely-related insect clades of Lepidoptera (e.g. Trichoptera, Antliophora, Hymenoptera) and some basal Lepidoptera clades are group-living, group-living could be the ancestral state and the evolution from group-living to solitary-living is likely. Also, the possibility that the evolution from aposematism to crypsis might happen cannot be excluded. It may also be possible that derived states can evolve back to the primitive states (backward evolution).

In Chapter 4, the coevolution of the binary states (solitary-living/group-living and cryptic/aposematic) is explored. Here I find that backwards evolution can happen (both from group-living to solitary-living and from aposematic to cryptic). The initial ancestral state is more likely to be the group-living state rather than the solitary-living state, although whether it is aposematic or cryptic is less certain. I also find that the evolution from group-living to solitary-living is more likely to happen in an aposematic lineage compared to a cryptic lineage and then followed by the transition from aposematism to crypsis. The reason for this could be that directly losing the group protection is risky for cryptic individuals, but initial evolution to aposematism could facilitate the transition from group-living to solitary-living so that the individuals are still protected by the aposematic defences in the process. Also, the aposematic state is still riskier than the cryptic state in a solitary lineage, so the evolution from an aposematic state to cryptic states is more likely to happen than the reverse.

In addition, I find that the transition rates from the solitary cryptic state to the two neighbouring states (group cryptic state and solitary aposematic state) is relatively low, so the solitary cryptic state is relatively more stable compared to the other states. After calculating the probability dynamics of the four binary states, I find that the solitary cryptic state has the highest probability value in the equilibrium regardless of ancestral state.

The findings in Chapter 4 are a little against intuition in at least two aspects. First, the ancestral state might not be solitary-living but is more likely to be group-living, so the previous studies about the evolutionary order of group-living and aposematism might be problematic. Second, there are coevolutionary dynamics in all the four binary states so, during the evolution, each of the four binary states has a certain probability of existing. Although the solitary cryptic state has the highest probability in the equilibrium, it is still possible to transit to other states at some point and then possibly evolve back later.

Chapter 5 studies the role of defences in macroevolution. As mentioned in Chapters 2 and 3, more effective defences can change the survival rates, and also change the selection force on the population, both of which are found to be associated with diversification rates. For one thing, higher survival rates can help the population to grow, and so a higher chance of diversification might follow [21, 22]. For another thing, different selection forces can make populations evolve in different directions, which is then followed by reproduction isolation and the generation of new species [23, 24, 25]. Ehrlich and Raven [26] have been cited many times for their hypothesis regarding the association between defences and diversification. There have been a few empirical tests for their hypothesis, although the number is still relatively limited. In Chapter 5, I used the same defence traits aposematism and group-living as used in Chapter 4, in order to add some new evidence to Ehrlich and Raven's [26] hypothesis.

I found that both aposematism and group-living are associated with faster diversification rates, which agrees with Ehrlich and Raven's [26] hypothesis, and especially, the association between aposematism and diversification rates is consistent with other empirical evidence [27, 22, 28]. However, the previous literature shows that the associations between chemical defences and diversification rates are less consistent, as the relations between the two can be either positive [29, 30, 31, 32, 33, 34], negative [35, 36], or non-existent [37]. This agrees

with our previous findings from Chapters 2 and 3, which show that earlier defences (e.g. aposematism) are more likely to be used and to be more effective at providing protection, therefore, earlier defences might be more likely to associate with higher diversification rates than later defences (e.g. chemical defences). Also, since the later defences (e.g. chemical defences) typically have higher variances than earlier defences, treating chemical defences using binary traits might have simplified their influence on diversification compared to the less variable earlier defences (e.g. aposematism). The association between group-living and diversification rates to my knowledge is new. Since group-living can either function as defence [38, 39, 40, 41, 42], or cooperative living and foraging [43, 44], and both of which can increase diversification rates, the association between group-living and diversification could be an extended area linked to Ehrlich and Raven's [20] hypothesis.

6.2 Limitations and future work

Both Chapters 2 and 3 use mathematical models to analyse the relationship between earlier and later defences in the sequential defence scenario. When using mathematical models, it is inevitable to use some assumptions either to simplify the questions or to focus on the key points or scenarios that are of interest. The model developed in Chapter 2 assumed that the whole population faces the same predation pressure, so the population evolves to have the same optimal defence strategy. I do this in order to find the optimal investment strategies for the populations in which defence strategies are found to be similar in individuals. Therefore, this model cannot be used to analyse a population which has a variation in the defence phenotypes, or in which the individuals have different defence strategies. However, I have tried to make the question as general as possible. As mentioned above, I have used the investment function in the most general form $f(x)$ compared to a linear form, and the defence numbers are a general n compared to two. In these ways, we do not need to think about whether a linear form function as well as whether two levels of defences will influence our results.

Compared to Chapter 2, Chapter 3 explores the variance in the mutation-selection balance in the population, so the predation pressures on defences are not assumed to be the same but stronger when the defences are further away from the ideal defence phenotype. In order to give the model a wide use, a normal form of mutation function and an exponential

form of selection function are used. I use a normal mutation function because, according to the central limit theorem, when a large number of independent, and identically-distributed events are observed, their mean tends to distribute normally. I use an exponential selection function because the cumulative distribution function of evenly-distributed events (Poisson Process) — that is defences are breached—not happening satisfies exponential distribution. Therefore, the model has wide uses in the real world when the event size is large.

The model I used to consider the mutation-selection balance in defence variances in Chapter 3 can also be applied to traits other than defences either to theoretically analyse the properties in trait variances or to empirically calculate the equilibrium variance values given the mutation and selection parameters.

There are two ways in which this research could be extended. First, we could include the competition or invasion of a second population with different characteristics (e.g. a different investment function, different damage costs caused by predators, or no defence investment at all but with a mimicry strategy). Second, we could include the coevolution between victims and their predators. So we can consider the interaction inside victims by their own and each other's population densities or abundances [45], their fitness values, their mimicry strategies [46], the character mutation rates in themselves or even to the other population, and so on; and we can consider the interaction between victims and their predators by each other's population density or abundance [47, 45], the mutation of defence characters and predation characters [13], and so on. In these ways, we could see the patterns of evolution of the defence strategies and distributions in the dynamics of the coevolution between victim populations and also between victims and predators.

Chapter 4 explores the coevolution of two defence traits (aposematic traits and group-living). It will be helpful to carry out the same study in other species, which would provide results for comparison with the present study in caterpillars, in order to see whether the similar pattern of dynamics can be observed in those species. Our method can be used to explore any other coevolved two or more than two categorical traits (e.g. defences traits or other traits) or genes (e.g. genetic network in genetic sequences [48]), and find out their possible ancestors, their future equilibrium and the dynamics across time. It can also be applied to predict the dynamics of populations' migration behaviour in several habitats.

In Chapter 5, I study the association between defences and diversification rates. Since the sample species in our dataset is relatively small compared to the number of species in the smallest clade that covers our dataset, I am not able to compare the diversification rates in the species with two defences (a) to the diversification rates in the species with one defence (b). If Ehrlich and Raven's [26] 's hypothesis holds, (a) could be larger than (b). This research can be carried out using clades that are more phylogenetically solved and whose dataset is more accessible than the Macrolepidoptera clade (e.g. fish clades).

In summary, this thesis aims to study the connection between defences in two important multiple defence scenarios— sequentially-deployed multiple defences and synergistically acting multiple defences. Different topics were explored: the evolutionary reason for multiple defences, the trade-off between defences, the distribution variances in defences, the coevolution between defences, and the association between defences and macroevolutionary diversification rates. Here the connections between earlier and later defences and between two synergistically acting defences are found to play important roles in the evolution of multiple defences, apart from the protective effect each defence has. Multiple defences are widely found in nature and also have applications in certain human practical areas (e.g. computer network defences), so understanding how they evolve interactively can be meaningful.

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